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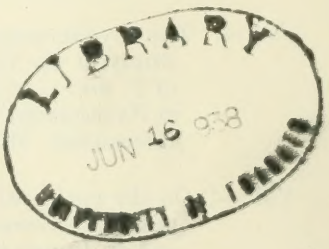
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AN ENQUIRY INTO THE ETIOLOGY, MANIFESTATIONS, AND PREVENTION OF PNEUMONIA AMONGST NATIVES ON THE RAND RECRUITED FROM "TROPICAL AREAS."

By G. D. MAYNARD, F.R.C.S.E.

In any discussion of the disease known as "Pneumonia" it is necessary at the outset to define clearly what is meant by this term. A few years ago a clear cut distinction was supposed to exist between lobar and catarrhal pneumonia. With the growth of bacteriology a tendency has arisen to class as pneumonic infections, all diseases which appear to be causally associated with the pneumococcus of Fraenkel. But, unfortunately, the term pneumonia is associated with a definite group of symptoms and physical signs, and such symptoms and signs *may* occur unassociated with the pneumococcus, while, on the other hand, this organism is frequently present in diseases which do not show the classical symptoms. If we attempt to record under the heading "Pneumonia" all diseases causally associated with the pneumococcus we are faced by grave, if not insurmountable, clinical difficulties; whereas, no survey of the effects of the pneumococcus can be considered complete which takes into account only one form of its manifestations.

Among the Natives recruited from tropical areas, who supply the bulk of the matter dealt with in this paper, Fraenkel's pneumococcus was frequently obtained in pure culture, by lung-puncture, from cases of lobar pneumonia. This organism has been also obtained from the sputum, and by lung-puncture, from some cases of broncho pneumonia. Further, it has been recovered in pure culture from abscesses unassociated with any lung symptoms, for example from a suppurating knee joint; and repeatedly from the cerebro-spinal-fluid. Finally, the pneumococcus was not infrequently recovered from the blood of patients suffering from lobar pneumonia. These observations show that amongst these Natives pneumococcal infections are as protean in their manifestations as they are known to be amongst Europeans.

The term "Pneumonia" will be used throughout this paper to mean the disease clinically known as lobar pneumonia when bacteriologically associated with the diplococcus of Fraenkel. We must not, however, lose sight of the fact that a survey of Pneumonia will be only a partial survey of the effects of the pneumococcus; but in the absence of a complete bacteriological examination in all cases of disease it is a limitation necessitated by our ignorance.

This paper is divided into the following sections:—

1. A consideration of the prevalence of pneumonia as influenced by race and country of birth. Death and attack rates, the case mortality, and the influence of season.

2. The clinical manifestations, course, symptoms, and gross morbid anatomy of the disease.

3. A consideration as to whether there be any evidence that the disease is infectious.

4. A consideration of the effect of prophylactic inoculation of a pneumococcal vaccine on the incidence and mortality of pneumonia.

5. General conclusions.

SECTION I.

A discussion of the prevalence of pneumonia as influenced by race and country of birth. Death and attack rates, the case mortality, and the influence of season.

Some of the Natives working on the mines of the Witwatersrand are recruited from districts north of lattitude 22° S. These Natives are colloquially termed "Tropical Natives"; this convenient expression we shall continue to use. The majority come from the Portuguese districts of Quilimane, Mozambique, Portuguese Nyassaland, and the district of Tete. It is impossible to divide these Natives into tribes. In recent years so much intermarriage has taken place that, except on very broad lines, tribal distinctions are not maintained. There are marked differences, however, between the Natives in the different territories in regard to their physique and liability to disease. These differences necessitate a separate consideration of the groups. Natives from some of these districts are apparently more heterogeneous than others, and this is particularly marked amongst the Quilimane Natives. For some purposes, therefore, they have been divided into four groups according to geographical sub-divisions of the district. All "Tropical Natives" employed on the Rand are recruited by the Witwatersrand Native Labour Association, and it is owing to the

courtesy of this Association that its valuable records have been placed at my disposal.

The Natives are recruited at their kraals, and marched from rest-camp to rest-camp until they reach a depôt at the port of embarkation. At these depôts they are housed and fed while awaiting the steamer which is to take them to Delagoa Bay. The ports of embarkation from North to South are as follows:—Port Amelia in Nyassaland, Mozambique, Angoche, Pebane for the district of Quilimane, and Chinde where the Tete Natives and Protectorate Independent Territorial Natives (the latter subsequently referred to as P.I.T.) are shipped. These Natives arrive at Chinde by boat viâ the Zambezi river. The voyage to Lourenço Marques occupies from five to twelve days. On arrival here the Natives are transferred to the train, and sent to the central low veld station of the Company at Ressano Garcia, where they are medically examined, detained for a few days, and then if passed as fit for mine work forwarded to the Rand. If the Medical Officer is not satisfied with their condition they are either sent home, or further detained at this place, to enable them to recuperate in a district with a climate similar to that of their homes. On their arrival in Johannesburg they are sent to the Association's compound, where they remain under close medical supervision for a further period of at least three weeks.* At the end of this time they are again medically examined, and if deemed fit for work are allotted to the mines, but if found to be unfit they are either returned to their homes or detained for a further period. It is during their stay in the Johannesburg compound that most of the records dealt with in this paper have been obtained.

The meaning of the terms used in connection with these Natives will now be readily understood. A Native not allotted to a mine at the end of three weeks with the gang to which he belongs is called a "detention"; if he be sent home a "reject." It is considered inadvisable to bring Natives from tropical districts on to the Rand during the winter, and, therefore, no recruiting is carried on in these areas during the four months April to July. The following tables, I. and Ia., show the numbers recruited from each area, with the repatriations, detentions, and deaths in the Johannesburg compound. The figures are obtained from the total number of Natives arriving in the seasons 1910 to 1911, and 1911 to 1912, and one-half of the recruiting season 1912 to 1913, that is, from August to 31st December, 1912.

*"Tropical Natives" are not permitted to work underground for a period of four weeks from the time of their arrival. There is no restriction to their doing surface work earlier, hence the period in the compound is on the average about three weeks

TABLE I.

Plain figures give actual numbers, italics rates per 1000.

Territorial origin.	Season.	Number of Natives recruited.	Detentions.	Repatriations.	Deaths in compound.
Mozambique ...	1910-11	5047	1368 *271·1*	174 *34·5*	220 *43·6*
,, ...	1911-12	4812	932 *193·7*	172 *35·7*	168 *34·9*
,, ...	1912	3412	637 *186·7*	71 *20·8*	129 *37·8*
,, ...	Total	13271	2937 *221·3*	417 *31·4*	517 *39·0*
Angoche ...	1910-11	3636	929 *255·5*	77 *21·2*	191 *52·5*
,, ...	1911-12	2517	518 *205·8*	362 *14·4*	161 *64·0*
,, ...	1912	1180	285 *241·5*	13 *11·0*	53 *44·9*
,, ...	Total	7333	1732 *236·1*	452 *61·6*	405 *55·2*
Quilimane ...	1910-11	2746	776 *282·6*	90 *32·8*	99 *36·1*
,, ...	1911-12	2106	467 *221·7*	90 *42·7*	58 *27·5*
,, ...	1912	1272	293 *230·3*	22 *17·3*	40 *31·4*
,, ...	Total	6124	1536 *250·8*	202 *33·0*	197 *32·2*

Territorial origin.	Season.	Number of natives recruited.	Detention.	Repatriation.	Deaths in compound.
Portuguese Nyassaland	1910-11	3574	652 *182·4*	61 *17·1*	85 *23·8*
,,	1911-12	2639	350 *132·6*	62 *23·5*	61 *23·1*
,,	1912	2808	454 *161·7*	29 *10·3*	78 *27·8*
,,	Total	9021	1456 *161·4*	152 *16·8*	224 *24·8*
Tete	1910-11	3077	315 *102·4*	19 *6·2*	16 *5·2*
,,	1911-12	1409	177 *125·7*	15 *10·7*	20 *14·2*
,,	1912	1585	232 *146·4*	3 *1·9*	11 *6·9*
,,	Total	6071	724 *119·2*	37 *6·1*	47 *7·7*
P.I.T.	1910-11	820	62 *75·6*	3 *3·7*	6 *7·3*
,,	1911-12	1221	129 *105·7*	10 *8·2*	23 *18·8*
,,	1912	200	36 *180·0*	1 *5·0*	4 *20·0*
,,	Total	2241	227 *101·3*	14 *6·2*	33 *14·7*
British Nyassaland Protectorate (B.N.P.)	1910-11	291	47 *161·5*	3 *10·3*	4 *13·7*
,,	1911-12	938	158 *168·4*	9 *9·6*	22 *23·5*
,,	1912	1			
,,	Total	1230	205 *166·7*	12 *9·8*	26 *21·1*

TABLE Ia.

SUMMARY.

Rates per 1000 in italics.

Territorial origin.	Number of Natives recruited.	Detention.	Repatriation.	Deaths in compound.
Mozambique .	13271	2937 *221·3*	417 *31·4*	517 *39·0*
Angoche ...	7333	1732 *236·1*	452 *61·6*	405 *55·2*
Portuguese Nyassaland ...	9021	1456 *161·4*	152 *16·8*	224 *24·8*
Quilimane ...	6124	1536 *250·8*	202 *33·0*	197 *32·2*
Maganja da Costa ...	2950	548 *185·3*	69 *23·3*	54 *18·3*
Lomwe ...	1620	590 *364·2*	60 *37·0*	74 *45·7*
Higher M'Locwe ...	1042	301 *288·9*	46 *44·1*	51 *49·0*
Lower M'Locwe ...	512	97 *189·5*	27 *52·7*	18 *35·2*
P.I.T. ...	2241	227 *101·3*	14 *6·2*	33 *14·7*
Tete ...	6071	724 *119·2*	37 *6·1*	47 *7·7*
P.N.P. ...	1230	205 *166·7*	12 *9·8*	26 *21·1*
Total ...	45291	8817 *194·7*	1286 *28·4*	1449 *32·0*

It will be observed that the rates vary considerably with the areas from which these Natives are recruited; and there is, as we shall see later, a high correlation between the number of "detentions" and the number of deaths.

The effect of dividing the Quilimane Natives into sub-groups is instructive. The death-rate for the whole group is 32·2 per thousand, which is practically the same as that found for the whole table. The rates in the sub-groups vary widely, Natives from the district of Maganja da Costa having a comparatively low death-rate, while those from the other districts have a much higher rate; so that the observed rate of 32 per thousand masks wide differences within the group.

Returning to Table Ia., it will be seen that the Natives with the highest death-rates are recruited from the districts of Angoche, Higher M'Locwe*, and Lomwe*, whilst those from the district of Tete have a much lower rate than that of any other group. The differences in the general death-rates are, as we shall see, largely due to variations in the prevalence of pneumonia, and from 44 to 82 per cent. of the total death-rate is due to the mortality from this disease. Excluding the group "diarrhœal diseases," Cerebro-spinal Meningitis is second to pneumonia in importance as a cause of death during the period of residence of these Natives in the W.N.L.A. Compound in Johannesburg. For this reason, and because its relative prevalence according to the territorial distribution of the Natives is not the same as that for pneumonia, the statistics of this disease have been included in Table II.

It is necessary to digress for a moment to enquire how far the death-rate from pneumonia may be regarded as an index to the attack-rate: that is, does a high death-rate from pneumonia necessarily indicate a high attack-rate? Clearly, if the case mortality were constant we could instantly deduce the one rate from the other; or given the death-rate, we could place the groups in the order of their liability to attack. In Column (10) of Table II. we see that the case mortality is not constant, and we therefore must determine whether a high death-rate is, or is not, associated with a high attack-rate. As the correlation of rates is unsatisfactory owing to a spurious correlation being thereby introduced, it is necessary to calculate the partial correlation by Professor Pearson's formula, which is as follows:—

If p = the population of the group, r = the number who recovered,

*It is worth recording that the head Recruiter reports that in these districts the Natives are exceedingly lazy, do very little cultivation, and are often, therefore, in a half-starved condition.

and d = the number who died, then the correlation of recoveries and deaths for a constant population is:—

$$_{p}r_{rd} = \frac{r_{ra} - r_{rj}\, r_{dp}}{\sqrt{1 - r_{rj}^2}\ \sqrt{1 - r_{dp}^2}}$$

In the case of pneumonia among the groups classified according to territorial distribution the value is:—

$$_{p}r_{rd} = \cdot 9045 \pm \cdot 0388$$

and there is, therefore, a high correlation between recoveries and deaths amongst those attacked with this disease. It will, consequently, make little difference whether we employ the attack-rate or the death-rate in estimating the prevalence of pneumonia among these groups. As in most enquiries the death-rate is the only factor obtainable this rate will be here used, since the results will be of more value for comparative purposes.

To return to the death-rate from pneumonia as given in Table II., and represented graphically in Map I. This map represents the rates occurring on the Rand amongst the Natives recruited from the different geographical areas, and does not refer to the conditions prevailing in the territories themselves. It is, of course, diagramatic, and if further sub-division of the Natives from the different districts had been possible the abrupt transition of the rates as shown would no doubt be considerably modified. That marked variations in the liability to this disease exist among the Natives recruited from these areas is plain, and it will be observed that the districts from which the highest rates are obtained is roughly a triangular area from Lake Chirua to the coast. The treatment of the Natives in regard to methods of recruiting, housing, feeding, etc., is practically uniform, except that the varying distance of their homes from the coast necessitates in some cases a lengthy march to the port of embarkation. That this is not a critical factor, however, is shown by this map, for the areas which yield the worst death-rates are not those farthest from the coast. Speaking generally, it appears that the Natives from the inland districts are, when residing on the Rand, less prone to the disease than those from the coastal areas. In the United States, when allowances are made for the fact that pneumonia is more prevalent in urban than in rural districts, we find that persons residing in the coastal districts are less subject to this disease than those living inland.[1] In regard to these Natives we do not know whether in their homes a similar condition obtains, but if further investigation were to show that this be so, a possible explanation of the reversal of this condition

on their arrival on the Rand would suggest itself; namely, that there had been a less rigorous selection amongst the Natives from the coastal areas.

Among other possible causes of these differences are:—

(1) That a general want of physique is correlated with a predisposition to all diseases.

(2) That certain tribes are particularly susceptible to pneumococcal infection, or

(3) That some factor favouring the spread of infection may be more marked in some groups than in others.

This last point will be dealt with more at length later, but it may be stated here that there is no evidence that a factor of infection has any influence in causing the variation in the death-rates. The Natives from the districts of Quilimane and Angoche are, at any rate on arrival at Delagoa Bay, of distinctly inferior physique to those recruited from Nyassaland and Tete; and also inferior, but to a less marked degree, to those coming from Mozambique. But, if general physical inferiority were the whole explanation, then we might expect that the death-rate from other diseases would show the same territorial distribution as the pneumonia rate.

A similarly prepared representation of the death-rate on the Rand from Cerebro-spinal Meningitis is shown in Map II. The figures used in preparing the maps, as well as those from which the attack-rates for the two diseases are calculated, are given in Table II. on page 10.

Here we see that the Natives from the three districts of Quilimane which show the highest death-rates from pneumonia, are, in respect to Cerebro-spinal Meningitis, among the best. The fact, however, that the numbers in these groups are smaller, must not be overlooked. Excluding these two diseases, the territorial distribution of the death-rate for all medical diseases gives a picture very similar to that of pneumonia. It is possible then, that the distribution of Cerebro-spinal Meningitis may be due to some peculiarity in method of spread, or in a special constitutional liability, and that general want of physique may still explain the variation in the death-rates from other diseases. We have not, however, at the present time, sufficient evidence to enable any suggestions to be formulated except in a tentative manner; that a marked and persistent difference in the tribal rates does occur, is, nevertheless, established.

It may be remarked that while some authorities state that the weaklings of a community are most prone to contract pneumonia, others assert that it is the robust who are most often attacked. A similar divergency in opinion is found amongst the medical practitioners engaged in Native work. This conflict of opinion need

TABLE II.

Tropical Natives recruited by W.N.L.A. during the period August, 1910, to December, 1912, inclusive.

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
				PNEUMONIA.								C.S. MENINGITIS.					
Territorial Origin.	Number of Recruits.	Total deaths.	Death-rate per thousand.	Cases.	Morbidity Rate per thousand.	Deaths.	Death-rate per thousand.	Per cent. of all deaths.	Case-Mortality per cent.	Other deaths.	Death-rate per thousand.	Cases.	Morbidity rate per thousand.	Deaths.	Death-rate per thousand	Case-mortality per cent.	Death-rate per thousand minus Pn. & C.S. Men.
Portuguese Nyassaland ...	9,021	224	24·8	290	32·2	106	11·8	47	36 6	118	13·1	80	8·9	42	4·7	52·6	8·4
Mozambique ...	13,271	517	39·0	674	50·8	244	18·4	47	36.2	273	20·6	134	10·1	92	6·9	68·6	13·7
Angoche ...	7,333	405	55·2	405	55 2	179	24·4	44	44·2	226	30·8	111	15 1	86	11·7	72·5	19·1
Maganja da Costa	2,950	54	18·3	77	26·0	27	9·1	50	35·1	27	9·1	13	4·4	10	3 3	77·0	5·8
Lomwe	1,620	74	45 7	89	54 9	44	27·2	57	49·5	30	18 5	3	1·9	3	1·9	100·0	16 6
Higher M'Locwe	1,042	51	49·0	71	68·2	31	29·8	61	43·7	20	19·2	3	2·9	1	1·0	33·3	18·2
Lower ,, ...	512	18	35 2	29	56·6	9	17·6	50	31·0	9	17·6	2	3·9	1	2 0	50·0	15·6
P.I.T.	2,241	33	14·7	51	22·7	27	12 0	82	52·9	6	8·5	1	0·4	1	0·4	100 0	8·1
Tete	6,071	47	7·7	94	15·5	27	4·4	57	28·7	20	3 3	8	1·3	5	0·8	62·5	2·5
B.N.P.	1,230	26	21·1	29	23·6	15	12·2	58	51·7	11	8·9	1	0·8	1	0·8	100·0	8·2
Total	45,291	1,449	32·0	1,809	39·9	709	15·7	49	39·2	740	16·3	356	7·9	242	5 3	68 0	11·0

occasion no surprise if such views are obtained merely from impressions rather than from carefully kept records; in other words, when the so-called "experiential method" replaces a scientific analysis of recorded facts.

Natives whose physical condition is poor are detained in the W.N.L.A. Compound beyond the average twenty-one day period, and, as before explained, are termed "detentions." If their condition is so bad, that, in the opinion of the medical officer, they are not likely to be fit for allotment to the mines within a reasonable time they are returned to their homes, and are termed "rejects." The physical condition of these Natives also varies from season to season, and at different times during the same season; the number of "detentions" and "rejects" from any group reflects, therefore, to a certain extent the condition of the whole group. By correlating either the general death-rate, or the pneumonia death-rate, with the number of "detentions" or "rejects," we shall obtain some measure of the association between the physical condition of the Natives and their liability to death.

Correlating all deaths (d) with "rejects" (n) for constant population (p), by the partial correlation formula, we find:—

$$_{p}r_{dn} = \cdot 6549 \pm \cdot 0715$$

and for all deaths and "detentions" (m),

$$_{p}r_{dm} = \cdot 8343 \pm \cdot 0381$$

Similarly the values for pneumonia deaths (e) and "rejects," and for all deaths and "detentions" are:—

$$_{p}r_{en} = \cdot 7764 \pm \cdot 0497$$
$$_{p}r_{em} = \cdot 4990 \pm \cdot 0941$$

Thus it is seen that in gangs where physically inferior "boys" preponderate both the total and the pneumonia death-rates are higher than where the reverse condition exists. Too much stress must not be placed on these results, as there is a considerable correlation between the "tribe" (or more correctly the Natives from a certain geographical district), and number of "detentions" or "rejects," so that we may be measuring the "tribal" liability rather than the physical condition of the gang: unless a difference in physique be the only factor of importance in this respect. The figures are of interest because they show that the physically inferior gangs have both a higher general death-rate, and a higher pneumonia death-rate, than those arriving in better condition; and the results, even interpreted on a racial basis, show that racial susceptibility is associated with a degree of physical unfitness. With the present incomplete data at

our disposal it would be unwise to be dogmatic on this question, but there are distinct indications that the physically unfit are more prone to pneumonia.

I have elsewhere shown[4] that the prevalence of pneumonia—taking the death-rate as a measure of the prevalence—is greatest amongst Natives during their first month of residence on the Rand, and that thereafter it progressively diminishes. With our present figures we can consider this problem as it relates to new arrivals during their first few weeks of residence in Johannesburg. With the exception of loss by death, the population in the W.N.L.A. Compound is stationary for all "Tropical Natives" for approximately three weeks. Dividing the first nineteen days into five-day periods, the number attacked in each period out of a total of 45,291 Natives, is as follows:—

TABLE III.

Day after arrival.	Number attacked.	Attack rate per cent.
0— 4	526	1·16
5— 9	389	0·86
10—14	314	0·69
15—19	275	0·61

Here again we see that the period of greatest risk is that immediately following arrival, the greatest number of cases being admitted to hospital on the first day. Thus, the liability commences suddenly and immediately after arrival, and decreases subsequently from day to day. This distribution is shown in Chart I., as also the theoretical curve, which is of the "J_1" type. It will be observed that the start is within the first twenty-four hours. The actual number of cases recorded is greater in the second twenty-four-hour period, but this is due to the fact that the first period is not really one of twenty-four hours, because the first day as determined from the compound books is often a period of only twelve hours, or even less. The marked reduction in attack-rate with the length of residence is a fact which must not be overlooked when we consider the evidence as to the probability of case to case infection. That this fall continues after the Natives are removed to the mines is also shown by other figures at our disposal. In the experiment

conducted with 8,123 Natives, which will be presently described, and is subsequently referred to as the "8000 experiment," the attack-rate month by month was recorded, and is here given in Table IV., and shown graphically on Chart II.

TABLE IV

Month after arrival.	Number attacked.	Attack rate per cent.
0	312	3·89
1	203	2·58
2	155	2·00
3	139	1·83
4	125	1·68
5	89	1·22

This Table also shows that amongst Natives recruited in "tropical areas" the maximum attack-rate occurs immediately on their arrival on the Rand, and gradually falls as the period of residence increases. The influence of season in producing variations in the attack-rate does not in any way enter into the problem in regard to the figures given in Table III.; and it is probably not of importance in Table IV., because the Natives composing the population dealt with in this table were recruited over a period from August to the middle of November, that is, from the coldest time during which recruiting from these districts is undertaken, up to, and well into the warm season. Chart III. shows the daily admissions from Cerebro-spinal Meningitis; and it is inserted here to show that this distribution is not similar in type to that found for pneumonia. There are some special features in this distribution which need not detain us here, but the distributions are clearly of different types.

It is of interest to note that a somewhat similar phenomenon to the fall in the attack-rate just discussed has been observed in Europe. In Nothnagel's Encyclopedia[5] we find the following sentence:—

"Gründler declares that the origin of the numerous pneumonic affections which he has noted in the garrison of Magdebourg from the 1st of October, 1873, to the end of June, 1874, was due less to the influence of the weather than to the arrival and education of recruits. . . . The complete strength of the garrison was 6,008 men, consisting of:—

Recruits, or men in 1st year of service: Pneumonia attack-rate	2·15%
Men in 2nd year of service: Pneumonia attack-rate	0·81%
Men in 3rd year of service: ,, ,,	0·67%
Men after 3rd year of service: ,, ,,	0·14%."

Aufrecht, the author of the article, considers that this predisposition of recruits is due to, " either unaccustomed or excessive muscular exertion, which may give rise to an enfeeblement of the entire muscular system, and, in connection with this, to an increased function of the sweat-glands." Further, after quoting some figures due to Riebe, he states:—" Any one who is not absolutely and wilfully blind cannot fail to see that the explanation of this state of affairs is to be found in the excessive demand on the powers of the young recruit and in the unwonted bodily exertion incident to the first year of service." These figures are not given in sufficient detail to enable us to judge whether they would if further sub-divided correspond in type to the distributions obtained from our Native returns; but if they did so correspond it would surely be only the " wilfully blind " who would be content to accept " either unaccustomed or excessive muscular exertion " as a sufficient explanation. In the case of the Natives referred to in Table III., excessive muscular exercise cannot explain the high attack-rate, nor could it account for the rate during the first month as given in Table IV. It has already been pointed out that all " Tropical Natives " have had, prior to their arrival on the Rand, a voyage of some ten days, followed by a detention in Ressano Garcia; and it is during their stay in the W.N.L.A. Compound in Johannesburg that the highest attack-rate from pneumonia occurs, and, therefore, before they commence the hard work incidental to mining. If, however, we had dealt with our data only in yearly groups, as in Gründler's returns, Aufrecht's suggested explanation might in this case also have appeared valid.

These figures of Gründler's are also quoted by Musser & Norris,[6] in support of a plea that occupation " plays an etiological rôle," and these authors further state " emigrants (*sic*) are more susceptible than Natives who have become acclimated." If this remark may be interpreted as meaning that immigrants suffer more from pneumonia than a native population of the same age and sex, it would have an important bearing on this problem. As an example of the contention that change of residence tends to increase the risk of contracting pneumonia they quote the case of the Lapps, but without giving their authorities or reproducing any figures, thus:—

" This is well exemplified in the case of the Lapps, who whenever

they move from the frozen north to a temperate zone almost invariably die of pneumonia within a short time." On the other hand, Sir W. Osler[7] writes as follows:—"Newcomers and immigrants are stated to be less susceptible than native inhabitants." This view is directly opposed to those given above, but in the absence of the data on which these views are based it is impossible to judge of their value.

The influence of season and climate must now be considered in so far as the available data permit. We have seen that for the territorial groups the attack-rate and death-rate are highly correlated, and we find from the figures in Table V., page 16, that this is also true for the monthly groups of this table.

The partial correlation for recoveries and deaths for constant population grouped according to months is:—

$$_{p}r_{rd} = \cdot 8739 \pm \cdot 0336.$$

Thus again we see that there must be a high correlation between cases and deaths; or in other words, the death-rate will serve as a close guide to the attack-rate. The problem, whether the case mortality is dependent on the rate of attack will be discussed later; but as will be shown, there appears to be little or no association between variations in the case mortality and in the prevalence of the disease.

In Chart IV. the death-rate for each month from August to the following April, both months inclusive, is shown for three successive recruiting seasons. The deaths have been recorded against the month in which the attacks commenced. It will be noted that in the season 1911 to 1912 the rate was highest in the colder months, and fell very noticeably in December and January, thus agreeing with an opinion quite commonly held that pneumonia is more prevalent during the colder months. In the season 1910 to 1911 the rate was highest in March and April, but no marked variations occurred from August to February. In the season 1912 to 1913 the maximum rates occurred during the warm weather, a reversal of the condition so marked in the season of 1911 to 1912. If we assume that other predisposing factors remained approximately the same, then from this evidence it would be difficult to state that season had any constant influence on the prevalence of the disease, although the tendency of the figures as a whole suggests that the prevalence decreases in the warmer months. This is shown graphically by the curve which represents the average rates for the three years.

We have no right, except hypothetically for the purpose of a first approximation, to assume that other factors have remained unaltered. For instance, we know that owing to a dry season in Tropical Africa in 1911 to 1912 there was a consequent shortage of crops, and it is possible that this factor may have had an influence on the Natives

TABLE V.

MONTH.	Average monthly number of recruits in Compd.	PNEUMONIA.					OTHER DISEASES.	
		Cases.	Rate per thousand.	Died.	Rate per thousand.	Case Mortality per cent.	Died.	Rate per thousand.
1910.								
August ...	1,293	43	33·3	11	8·5	25·6	32	24·7
September ...	1,864	69	37·0	27	14·5	39·2	98	52·6
October ...	2,077	61	29·4	22	10·6	36·1	78	37·6
November ...	2,141	73	34·1	36	16·8	49·3	36	16·8
December ...	2,375	68	28·6	28	11·8	41·2	18	7·6
1911.								
January ...	1,829	41	22·4	19	10·4	46·4	22	12·0
February ...	1,827	47	25·7	22	12·0	46·7	31	17·0
March ...	2,200	135	61·4	54	24·5	40·0	53	24·1
April ...	1,722	94	54·6	33	19·2	35·1	1	0·6
May	343	3	8·7	0				
June	69	0						
July	2	0						
August ...	939	143	152·3	48	51 1	33·6	48	51·1
September	2,040	219	107·4	72	35·3	32·8	16	7·8
October .	1,609	143	88·9	43	26·7	30·0	6	3·7
November .	816	30	36·8	9	11·0	30·0	16	19·6
December ...	1,085	26	24·0	6	5·5	23·0	8	7·4
1912.								
January ...	936	16	17·1	5	5·3	31·0	19	20·3
February .	1,669	39	23·4	17	10·2	43·6	20	12·0
March ...	1,993	84	42·1	34	17·1	43·8	74	37·1
April	1,749	85	48·6	47	26·9	55·4	19	10·9
May	258	1	3·9	1	3·9	100·0		
June	20	0						
July	4	0						
August ...	1,552	96	61·9	39	25·1	40·6	41	26 4
September ...	2,251	81	36·0	35	15·5	43·2	2	0·9
October ...	1,448	40	27·6	25	17·3	62·6	32	22·1
November ...	1,751	73	41·7	35	20·0	48·0	11	6·3
December .	1,718	99	57·6	41	23·9	41·5	59	34·3
1913.								
January ...	2,484			62	25·0		73	29·4
February ...	2,290			49	21·4		28	12·2
March	2,226			26	11·7		17	7·6
April	1,170			11	9·4			

arriving in the season of 1912 to 1913; again, a large proportion of Natives who arrived between November, 1911, and November, 1912, were receiving prophylactic inoculations of pneumococcal vaccine.

Some additional information on this question of seasonal prevalence can be obtained by a consideration of certain returns applying to Natives at work on the mines. Through the kindness of Dr. G. A. Turner, I have been able to obtain figures giving the average monthly number of Natives employed on the mines, with the corresponding deaths from pneumonia, for five years; subdivided according to the territorial areas from which they were recruited. In Chart IV. the death-rates per month for a five-yearly average are shown. In Chart V. the death-rate occurring in each month has been plotted in percentages of the total rate. Four distributions obtained from data relating to European populations in different parts of the world are also shown for comparison. The two distributions obtained from figures relating to populations in the Northern Hemisphere have been plotted by reading July for January, and so on, so that the seasons shall correspond.

It will be noticed that the monthly fluctuations in the Native distributions are not so marked as those in the European distributions, and that Natives from British territory show a greater seasonal variation than those from Portuguese territory. Nevertheless, all groups show lower rates during the warmer months. In regard to British Central African; Mozambique; and Zambezi, Quilimane, and Tete Natives; it must be remembered that no new arrivals are being allotted to the mines from May to nearly the end of August, and this factor in itself will, as we have seen, tend to reduce the death-rate observed in these months. Monthly fluctuations among "Tropical Natives" are, however, much more marked; this is partly accounted for by the fact that the rates are calculated from smaller numbers.

Summing the whole of this evidence, we may, I think, conclude that pneumonia tends to be definitely more prevalent during the colder months. Fluctuations due to other causes, more especially amongst the "Tropical Natives," may, however, to a large extent mask the effect of season when small groups are dealt with. Further, the effects of seasons are probably dependent on the meteorological conditions with which they are associated; for instance, the temperature or rainfall of the May of one year might correspond more closely to that of the June than the May of a preceding, or following year. The agreement, therefore, between the rates and changes in meteorological conditions might be greater than that observed in the monthly charts. Alterations of weather conditions are, no doubt, highly correlated with season; these facts, nevertheless, must be borne in mind when

attempting to interpret the meaning of the foregoing data.* Cold or a fall in temperature is the constant factor of the winter season in all parts of the world. In the interior of South Africa the winter season is the dry and dusty season, but in Northern Europe it is the damp season.

It is interesting to recall that this view of the influence of season on the incidence of pneumonia was realised by the Greek physicians. Two of the aphorisms of Hippocrates show this. Sec. III., No. 19: "All diseases occur at all seasons of the year, but certain of them are more apt to occur and be exacerbated at certain seasons." In the following aphorisms he proceeds to deal with those diseases prevalent in Spring, Summer, and Autumn, and No. 23 reads as follows: "Of Winter, pleurisy, *pneumonia*, coryza, hoarseness, cough, pain of the chest, pains of the ribs and loins, headache, vertigo, and apoplexy."[9]

To return to a consideration of the relationship of the attack-rate to the case mortality; this is of some importance from a statistical point of view, as well as, in its physiological aspect. It must be studied first of all in regard to the tribal distribution, and then in respect to season. The problem may be stated in this way; is a "tribe" that is especially prone to pneumonia liable to a higher case mortality than a "tribe" less liable to attack, or the reverse? In other words, does an increased liability to attack imply an increased liability to death when attacked? If this be so, then by using death-rates to judge of the prevalence of attack we should be exaggerating any differences in the attack-rates that may be present. The co-efficient of correlation for attack-rate and case mortality, in the case of the territorial distribution, is:—

$$r = 3895 \pm \cdot 1809$$

but this value is unfortunately not a safe guide owing to mathematical difficulties, and to the introduction of a spurious correlation due to the peculiar nature of the problem. If r = recoveries and d = deaths, the case mortality as usually expressed $= 100 \frac{d}{r+d}$. If the case mortality is constant, then $\frac{d}{r+d}$ is constant, and d is proportional to r. If $\frac{d}{r+d} = x$ then $d = r\frac{x}{1-x}$. The partial correlation, therefore, of r with

* It will be noted that the seasonal prevalence of pneumonia which is similar in the figures obtained from Europe, America, Australia, and South Africa, markedly diminishes with the advent of warmer weather, and increases again as the winter approaches. This phenomenon is not consonant with the theory which has recently been suggested by some, that the house fly is an important factor in the dissemination of this disease.

d for x constant will be unity. In so far as the value found falls short of unity it will be a measure of the variability of x, i.e., the case mortality.

We have already found the partial correlation:—

$$_{p}r_{rd} = \cdot 9045 \pm \cdot 0388$$

and this when considered with its probable error leaves very small grounds for any assertion that the case mortality varies, to any appreciable extent, with the attack-rate.

Turning now to seasonal data, we find, correlating directly the attack-rate with case mortality:—

$$r = -\cdot 2143 \pm \cdot 1342.$$

The partial correlation of recoveries with deaths for constant population has been shown to be:—

$$_{p}r_{rd} = \cdot 8739 \pm \cdot 0336.$$

Here, then, it would seem that there is rather more justification for assuming that the case mortality is not, within the limits of random sampling, a constant.

The crude correlation for the rates in these two instances gives values which, when considered in relation to their probable errors, cannot be regarded as significant, and are of unlike sign.

It would appear, therefore, that the case mortality certainly does not bear any close relationship to the attack-rate; and the figures before us suggest that an increase in the attack-rate is not associated with an increase in the case mortality from the disease.

SECTION II.

The Clinical Manifestations, Course, Symptoms, and Gross Morbid Anatomy of Pneumonia.

The clinical signs and symptoms of an uncomplicated case of pneumonia in a "Tropical Native" do not differ materially from those in a European. There is, however, a marked difference in temperament; the Native being apparently much less sensitive to the discomfort of illness than the European. As a result he frequently remains at work when seriously sick unless under careful European supervision. Although the onset of pneumonia is usually sudden and accompanied by high fever, yet not infrequently the Native will fail to report sick if left to himself.

Out of 975 cases which recovered, the fever terminated by lysis on 503 occasions (51·6 per cent.); by crisis on 338 occasions (34·7 per cent.); and its termination was atypical—i.e., could not satisfactorily be classified under either of the previous headings—in 134 cases (13·7 per cent.). In the European statistics which I have been able to consult the manner in which the fever terminated is usually classed into only the two groups, crisis or lysis; and no doubt some of those cases which are here grouped as atypical would appear in the lysis group, while others would possibly have been classed as crisis. The term "crisis" as used in this classification is understood to denote a sudden and continued fall of temperature to the normal, which is reached within about thirty-six to forty-eight hours of the commencement of the fall. Where a sudden fall has been followed by an intermittent temperature for several days, as is not uncommon, the case has been classed as atypical, as also cases in which the temperature has been intermittent from the commencement, apyrexial cases, and so on. Among the 134 cases classed as atypical 23 were afebrile, and four were not admitted to hospital until after the temperature had fallen to normal.

To consider first those cases which terminated by *crisis*. Out of a total of 378 cases there were 43, or 11·4 per cent., that died after the temperature became normal; 25 of these were, so far as a macroscopic post mortem examination could determine, unaccompanied by any pathological process other than a pneumonic consolidation in the lungs. Twelve of these deaths occurred within 24 hours of the time the temperature reached the normal; the remainder were distributed as follows:—One on the third, three on the fourth, fifth and seventh respectively, two on the ninth, and one on the seventeenth day.

Chart VI. shows the distribution of cases according to the day after admission on which the temperature reached the normal, which, for practical purposes, we may term the *day of crisis*. For comparison a similar distribution of 4,718 cases recorded by Musser & Norris is shown, and also a series of 873 cases by Aufrecht.[5] The chief constants of these distributions are given in the following table:—

TABLE VI.

		"Tropical Natives."	Musser & Norris	Aufrecht.
Mean	...	5·3519 ± ·0849	6·6045 ± ·0234	6·7211 ± ·0596
Mode	...	4·5733	5·8797	5·9871
σ	...	2·4483 ± ·0600	2·3450 ± ·0165	2·6088 ± ·0421
β_1	...	0·2968 ± ·0954	0·5725 ± ·0661	0·5684 ± ·1833
β_2	...	3·2219 ± ·2657	4·2656 ± ·2622	4·4898 ± ·8972
Type	...	I.	IV.	IV.
Number	...	378	4718	873

Here a word of caution is necessary, a death occurring in the first twenty-four hours is considered as occurring on the first day, a death occurring in the middle of the sixth day would correspond to a position on the chart of 5·5 on the abscissa; thus the mean day of crisis is given as 5·35 days, that is, it falls in the sixth day. A reference to Chart VI. will show that the two European distributions are similar in type and position; and considering the value of their constants in relation to the probable errors their similarity suggests that these curves closely represent the usual distribution for "crisis" in pneumonia amongst Europeans in the Northern Hemisphere. Both distributions are definitely leptokurtic, while that obtained from the Native data is, within the limits of errors of sampling, mesokurtic. The standard deviation of all three distributions is approximately the same. The most marked difference between the Native and European curves is, that the former may be described as having been bodily moved to the left by about a day and a half; this, if it be significant of a difference in the type of disease, would mean that the course of the illness is, on the average, a day and a half shorter. It will no doubt occur to the reader that this difference is possibly due to Native admissions to hospital not taking place until about thirty-six hours after the commencement of the illness, but I do not think this is the probable explanation. Although a Native is often slow to voluntarily report himself sick, yet in the W.N.L.A. Compound the police

boys inspect all compound rooms several times a day in order to discover and report any "boy" who appears sick; and, as the onset of this disease is usually more or less sudden and severe, it seems hardly likely that we must allow an average of thirty-six hours between the onset of symptoms and admission to hospital. Further, the greatest number of admissions takes place within the first twenty-four hours of the arrival of the Natives on the Rand. During the journey from Ressano Garcia they are under the supervision of a competent European, who sends any sick "boy" to hospital immediately on arrival. If it should be thought that the large number of admissions occurring immediately after the arrival of the Natives on the Rand be due to an accumulation of cases that developed the disease prior to their arrival, then the case mortality and length of illness after admission to hospital might be expected to throw some light on such a proposal. These problems are considered on pp. 25 and 34, and it will be seen that there is no evidence to support such a suggestion.

If we can dismiss as unfounded the supposition that the alteration in the position of the Native curve be due to the delayed admission of cases to hospital, then we have evidence that pneumonia runs a shorter course amongst "Tropical Natives" than amongst Europeans in the Northern Hemisphere.

Turning now to an examination of the cases classed under the heading *lysis*. The distribution of these cases, grouped according to the day on which the temperature reached the normal, is shown in Chart VII. It is more difficult to obtain data from European sources for comparison. Aufrecht in Nothnagel's Encyclopedia[5] writes:—"It is well in all cases in which the drop in temperature occurs by lysis to look for the existence of a complication. Even if it is not proper in such cases to exclude a true pneumonia and accept pneumonic relapses as the most frequent cause, nevertheless, complications may be the cause of a fall in temperature by lysis." The only figures he quotes are the following from Wagner:—"In the years 1881 to 1883 he (Wagner) saw among 199 pneumonias only 115 terminate by crisis (26 by protracted crisis), and 84 by lysis." That is 42 per cent. of these cases ended by "lysis," and 45 per cent. by what would in this paper have probably been called "crisis." Of the 975 cases in our records 52 per cent. terminated by "lysis," and 35 per cent. by "crisis." I have been unable to find any figures from European cases which enable a detailed comparison with the Native cases to be made. Reference to the chart will show that the commonest day for the temperature to reach normal by "lysis" is approximately two days later than that for "crisis"; that is, the mode is at 6·5 days as against 4·3 in the "crisis" distribution.

The constants of the "lysis" distribution are given in the table below:

TABLE VII.

Constants of distribution when termination is by Lysis.

Mean	...	...	...	$7 \cdot 4609 \pm 0 \cdot 0802$
Mode	...	...	...	$6 \cdot 5028$
σ	...	...	...	$2 \cdot 8234 \pm 0 \cdot 0567$
β_1	...	...	...	$0 \cdot 2924 \pm 0 \cdot 0724$
β_2	...	...	...	$3 \cdot 1272 \pm 0 \cdot 1982$
Type	...	...	...	I.
Number	...	...	...	564

Both the "crisis" and the "lysis" distributions are very closely similar in regard to their variability, skewness, and kurtosis, the difference in the positions of the mean and mode is, however, marked. This shifting of the mode may be a measure of the extra time required for the temperature to fall to normal, and suggests that if we had been able to date the records of the cases from the commencement of the fall in temperature the two distributions might have been almost identical. If this be so, the explanation of the cause of a fall of temperature by "lysis" as suggested in the extract quoted above would not appear to be satisfactory, at any rate, if applied to the pneumonia of "Tropical Natives."

There are, however, appreciable differences between the types of the disease as met with among these Natives and the European examples quoted; first, in the duration of the febrile period, and secondly, in the smaller percentage of all cases which terminate by "crisis."

If we form a table by entering each case according to day of termination by crisis, and day of admission to hospital after arrival in compound, we find that the coefficient of correlation is zero, for

$$r = -\cdot 0035 + \cdot 0346,$$

and thus we may conclude that Natives attacked immediately on arrival are neither more nor less likely to have a prolonged illness than those attacked later. This value also indicates that attacks do not commonly commence before arrival in the compound, for if they did, we might reasonably expect that the length of the febrile stage in hospital would be of shorter duration; unless, indeed, we assume that the exposure and lack of proper treatment before admission increased the severity of the attack, in which case the mortality among the earlier cases should be higher than the later, which it is not.

The same statements apply to the group of cases in which the pyrexial stage terminates by "lysis." From a similarly constructed table the value of the correlation is found to be

$$r = -\cdot 0258 \pm \cdot 0282.$$

The ratio of cases in which the fever terminated by "lysis" to those terminated by "crisis" is shown in Table VIII., for periods of five days.

TABLE VIII.

Ratio of cases terminating by lysis to those by crisis.

Days ...	Total	0-4	5-9	10-14	15-19	20-24	25-29	30 and over.
Crisis ...	378	122	81	59	46	33	7	30
Lysis ...	564	167	127	99	95	37	15	24
Ratio ...	149	137	157	168	203	112	214	80

We have already referred to the hypothesis that a termination by "lysis" indicates a different type of attack, and have quoted one author's opinion that a fall of the temperature by "lysis" probably indicates the presence of some complication; we can, therefore, proceed to examine this problem from another point of view. The duration of convalescence after the temperature has reached the normal will enable us to form some opinion as to the degree of severity of the attack, and also as to the probable presence of complications. Table IX. gives the chief constants of the distributions for recoveries from pneumonia after termination of the fever by "crisis" and "lysis" respectively, according to the number of days spent in hospital after the temperature reached normal. Cases in which a second attack followed the first before discharge from hospital have been omitted from both distributions.

TABLE IX.

Constants of distribution for days in hospital after the febrile stage.

	Crisis.	Lysis.
Mean ...	$10 \cdot 9697 \pm 0 \cdot 1906$	$10 \cdot 2889 \pm 0 \cdot 1589$
Mode ...	$5 \cdot 3337$	$5 \cdot 4325$
σ ...	$5 \cdot 1336 \pm 0 \cdot 1348$	$4 \cdot 9858 \pm 0 \cdot 1124$
β_1 ...	$1 \cdot 8809$	$2 \cdot 0976$
β_2 ...	$5 \cdot 0645$	$5 \cdot 5951$
Start ...	$5 \cdot 1036$	$4 \cdot 8854$
Number ...	330	488
	Type I.	Type I.

There is no significant difference between these two distributions, and any suggestion that a fall of temperature by "lysis" is due to complications which are likely to delay convalescence is, as regards these figures, quite unjustified. Further, we may remark that there is only a very small correlation between the day of illness on which "crisis" took place and the length of time spent in hospital after the temperature became normal; that is, patients who have a short attack of fever do not seem on the average to convalesce more rapidly than those in which the pyrexial stage lasts longer. It may here be remarked, that usually owing to pressure of cases for admission the patients are discharged from hospital as soon as it is safe to do so. In the W.N.L.A. Hospital there is no routine period of retention after the febrile stage is over. The absence of any fixed period of retention is shown by the variability of the distributions—as shown in Table IX. by the standard deviations. The pressure of new cases sometimes necessitates the discharge of cases to the convalescent rooms before they would otherwise have been so transferred, and it is probable that those whose attack is of shorter duration would, other things being equal, be the first to be sent out, and thus this factor may possibly account for the small correlation actually found. The values are:—

(1) Day on which temperature reached normal by "crisis" with days spent in hospital after normal temperature was reached.

$$r = \cdot 0823 \pm \cdot 0369.$$

(2) Day on which temperature reached normal by "lysis" with days spent in hospital after normal temperature was reached.

$$r = \cdot 1194 \pm \cdot 0301.$$

It would seem then that the fall of temperature to normal marks a definite stage in the disease, probably the termination of the infective process, and that thereafter the stage of convalescence is very little influenced by the length of the previous pyrexia.

It is of some importance to try and determine on rational grounds the stage at which we may consider the disease to have ended, in order that we may be able to distinguish between an extension of an established pneumonic process and a new attack.

In considering the question of the termination of the attack in the light of modern theories of acquired immunity, the investigation of second attacks is of considerable interest and importance. We must first consider what meaning we shall attach to the terms "second attack," "recurrence," and "extension of the disease." Is there any essential difference in meaning between these terms?—and if so, in what does it consist? It is quite easy to understand that a gradually spreading pneumonic process in a lung, where we may assume that immunity has not yet been established, is totally different to a new infection occurring after a period of return to normal health; but is

there any essential difference in the pathological process involved in a gradually spreading consolidation, and a more or less sudden affection of a hitherto unaffected lobe during the course of the febrile period? If we assume that these two conditions are but different manifestations of a similar process then we must enquire at what stage in the course of the disease, or at what stage during or after convalescence, we are entitled to consider the reappearance of symptoms as a "second attack." It will be noticed that the word "recurrence" is not used, because this word does not seem to denote a process essentially different from a second attack, unless it refers to a recrudescence of the original infection. In this case, the time factor alone can separate it from "extension of the disease," and any such difference must of necessity be, it seems to me, purely arbitrary. The term "recurrence" seems to be applied indifferently to two probably separate conditions, and in this category may be found cases belonging to both the above classes; if this be so, then the use of this word merely serves further to obscure an already somewhat difficult problem. It will, I think, be readily admitted that if the length of time after the first invasion be the only difference between a "recurrence" and a "second attack" it will help to simplify our ideas if we drop the former term; if on the other hand an essential difference between the processes exist we must reintroduce it when the difference has been scientifically defined.

In this connection the leading text-books of medicine do not, unfortunately, give us much assistance. Sir W. Osler,[7] under the heading *Previous Attack*, writes:—"No other acute disease recurs in the same individual with such frequency. Instances are on record of individuals who have had ten or more attacks. The percentage of recurrences has been placed as high as fifty. Netter gives it as thirty-one, and he has collected the statistics of eleven observers who place the percentage at 26·8. Amongst the highest figures for recurrences are those of Benjamin Rush, 28, and Andeal, 16." Under the heading *Relapse*, we find the following remarks:—"There are cases in which from the ninth to the eleventh day the fever subsides, and after the temperature has been normal for a day or two a rise occurs and fever may persist for another ten days or even two weeks. Though this might be termed a relapse, it is more correct to regard it as an instance of an anomalous course of delayed resolving. Wagner, who has studied the subject carefully, says that in his large experience of 1,100 cases he met with only three doubtful cases. When it does occur, the attack is usually abortive and mild." The meaning of this quotation appears to me obscure. In the first extract "previous attacks" are spoken of as "recurrences," and we must therefore regard these terms as synonymous; further we are told that

such events occur frequently. A "relapse" is apparently some condition of rare occurrence, and the context makes it impossible to give the word its usual meaning, as applied to the patient in contradistinction to a phase of the disease.

Aufrecht, in the article "Inflammation of the Lungs,"[5] writes:—"Also in adults pneumonias may occur repeatedly. . . . According to Riesell's experiences, among every hundred pneumonias almost regularly 50 represent first attacks, 32 second, 15 third, while the remaining 3 represent a fourth, or even more frequently repeated attack." Musser & Norris[6] state:—"Among the writer's cases collected in reference to this subject 1,615 out of 11,811 had previous attacks (13·6 per cent.), and of 246, 189 were in the second, 33 in the third, 18 in the fourth, 3 in the fifth, 1 in the sixth, 1 in the eighth, and 1 in the ninth attack."

These extracts show that "recurrences," and "second attacks" are well recognised conditions, and not uncommon; but no definition of the differences, if any be implied by these terms, is given.

In any case of pneumonia the consolidation may be limited to one lobe, but not infrequently two or more lobes are affected. If the neighbouring lobe is most commonly involved by a direct extension of the infective process we ought to find two adjacent lobes more often attacked than two lobes not in direct contact. A reference to Table X., page 28, shows that there were 247 cases in which two lobes only were affected, and gives the distribution of the pairs affected. The data in this table were obtained entirely from post mortem examinations. Out of 213 cases where only one lobe was affected the percentage distribution was as shown in Column (1). Assuming that the first lobe of the affected pairs followed a similar distribution, and that of the remaining four unattacked lobes the rate of those attacked would be in a similar ratio, we can calculate the distribution of pairs to be expected. It is given in Column (2) to the nearest unit. The observed distribution is shown in Column (3), and the differences in Column (4).

We see that adjacent lobes, that is the upper and middle, and middle and lower, on the right side, and the upper and lower on the left, are more frequently both attacked than they ought to be on our assumption, while one lobe of each lung, excluding both lower lobes, occurs much too seldom. It may quite fairly be objected, that considering the anatomical structure of the right lung, we are not justified in regarding the middle lobe as an anatomical entity, and that it should be considered merely as a portion of the upper lobe; for much of it is directly continuous with this lobe. If then we recalculate our table and add to the total number of pairs formed by the upper and lower, and middle and lower, the group "all three right lobes" we obtain by a similar process the results shown in Table XI., page 28.

TABLE X.

		Distribution per cent. of lobes affected when only one lobe is involved. (1)	Expected Distribution for pairs. (2)		Observed Distribution for pairs. (3)	Difference (Column 3 minus Column 2.) (4)
R.	Upper ...	24·0	Upper & Middle	4	16	+ 12
R.	Middle	2·8	Lower ,, ,,	5	16	+ 11
R.	Lower ...	30·4	R. Upper & Lower	50	37	— 13
L.	Upper ...	6·7	L. Upper & Lower	16	68	+ 52
L.	Lower ...	36·1	Both Upper	9	6	— 3
			,, Lower	82	83	+ 1
			Unpaired lobes in two lungs	81	21	— 60
			Total ...	247	247	

TABLE XI.

	Expected distribution for pairs.	Observed distribution for pairs.	Difference.
R. Upper and Lower	85	151	+66
L. Upper and Lower	17	68	+51
Both Upper ...	16	6	–10
Both Lower ...	92	83	– 9
Unpaired lobes in two lungs ...	119	21	–98

TABLE XII.

	Expected distribution for pairs.	Observed distribution for pairs.	Difference.
R. Upper and Lower	86	151	+65
L. Upper and Lower	19	68	+49
Both Upper ...	18	6	–12
Both Lower ...	90	83	– 7
Unpaired lobes in two lungs ...	116	21	–95

Again we see that an affection of adjacent lobes is in very marked excess of the theoretical number. From both tables we may conclude —accepting the above hypothesis as correct—that at least one-third of the infections of second lobes are due to direct extension of the disease from lobe to lobe. If we further assume that the reverse of the proposition is also true, and that in two-thirds of the cases the second lobe becomes affected by a new focus of the disease (arising from some cause other than direct extension), we must also assume that this new centre might appear in the lobe which had been first affected. Reconstructing our table on this assumption, we obtain the results shown in Table XII., page 28.

This again demonstrates the same phenomenon, and we have, therefore, some justification for assuming that the infective process may spread by direct extension from lobe to lobe.

Let us provisionally consider all cases in which the temperature rises after it has been normal for twenty-four hours or more, when the rise is accompanied by a reappearance of the signs and symptoms of pneumonia, to be "second attacks." The cases have been divided, (Tables XIII. and XIV.) according to the length of the period between the end of the first attack, as previously defined, and the commencement of the second; so that if this definition does not satisfy the reader he can see how great, or how little, a difference will be made by excluding such cases as he may desire from the distribution, and considering them as recrudescences of the first attack.

When attempting to estimate the frequency of second attacks considerable difficulty arises in determining the size of the population in which the second attacks arose, and only in the "8,000 experiment" can this be obtained with approximate accuracy for a period exceeding one month. From the number attacked during each month of residence on the Rand must be deducted (1) the number of deaths during first attacks, (2) the number of deaths from other causes, and (3) the number repatriated. The resulting populations are given in Table XIIIa. This table is to be read thus: In the first row, and first column, is found the number 168, which indicates that there were 168 Natives who recovered from an attack of pneumonia contracted during their first month of residence on the Rand, and who remained under observation for thirty days after recovery. In the first row, and second column, the figure 151 occurs, which indicates that the population from which second attacks were possible during the second period of thirty days from the end of a first attack contracted within a month of arrival, had been reduced by seventeen. In Table XIIIb. the actual number of second attacks is given, according to the date of occurrence of first attack, and the length of time after recovery before the second was contracted. In Table XIIIc the rates per month per thousand of population are given.

TABLE XIII. A.

Population, i.e., number of "boys" exposed to risk of second attack.

Month after arrival in which 1st attack occurred.	Months after termination of 1st attack.						
	1	2	3	4	5	6	Total.
1st	168	151	139	127	115	104	804
2nd	136	133	123	115	113		620
3rd	92	85	80	75			332
4th	53	48	47				148
5th	76	73					149
6th	40						40
Total	565	490	389	317	228	104	2093

TABLE XIII. B.

Number of second attacks.

Month after arrival in which 1st attack occurred.	Months after termination of 1st attack.						
	1	2	3	4	5	6	Total.
1st	12	2	6	3	5	4	32
2nd	1	5	4	1	0		11
3rd	5	2	3	0			10
4th	2	0	0				2
5th	2	2					4
6th	1						1
Total	23	11	13	4	5	4	60

TABLE XIII. C.

Rates per thousand.

Month after arrival in which 1st attack occurred.	Months after termination of 1st attack						Average Monthly Rate.
	1	2	3	4	5	6	
1st	71	13	43	24	43	38	40
2nd	7	38	33	9	0		18
3rd	54	24	38	0			30
4th	40	0	0				14
5th	26	27					27
6th	25						25
	41	22	33	13	22	38	28·7

It will be noticed that there is apparently a tendency for the rates to fall as the length of residence on the Rand increases, a phenomenon also observed for first attacks. Further, although the rates are irregular owing to their having been calculated from small numbers, there seems to be a tendency for second attacks to become less common as the interval after the first increases. It must be remembered that the numbers in column (1) of Table B. must be too small, as second attacks, which occur before discharge to work, were not notified by the mine authorities; and there will, therefore, be few or no cases in the mine records occurring within fourteen to twenty-one days after the first attack. The mean monthly attack-rate for the six-month period is 28·7 per thousand, which, as we have just seen, must be somewhat too low for the period dealt with in the tables.‡ The mean monthly attack-rate for first attacks out of the whole population for the same period is 22·3 per thousand.

Out of 1,129 Natives who recovered from pneumonia in the W.N.L.A. Compound Hospital eighty had a "second attack" within thirty days from the termination of the pyrexial period of the first attack; the distribution in days after first attack is shown below:—

TABLE XIV.

Day after termination of fever of first attack	Number of cases of "second attacks."	
0	1	
1	4	
2	12	30
3	4	
4	5	
5	4	
6—10	17	
11—15	14	
16—20	12	
21—30	7	
Total	80	

The population throughout this period remained practically constant, and we therefore find a rate of seventy per thousand for second attacks occurring within thirty days of the end of the first attack, as against a rate of forty per thousand for primary pneumonias

‡ Owing to the construction of the table, the rates for the columns will be progressively too high as the period increases; for the attack-rate for second attacks apparently decreases with increase both in period of residence and length of interval after the first attack. This defect in the table is unavoidable, as the period of observation was limited to six months. Further, in reference to this table the remarks made in connection with the effect of prophylactic inoculation must be borne in mind (see p. 89).

originating within about a month from the date of arrival of the Natives in Johannesburg.

These figures, showing that amongst "Tropical Natives" in the W.N.L.A. Compound "second attacks" amongst the attacked are more frequent than "first attacks" among the whole population, may indicate that one attack predisposes to a second; or that we are dealing with a particularly susceptible population selected as such by the first attacks. The question is one of considerable difficulty, and in considering it one must not lose sight of the fact that "second attacks" appear to become less common as the period after the first increases; if this be a rule then it would lend support to the suggestion that the first attack actually predisposes to the second. The mechanism of recovery would, however, be difficult to understand on the basis of modern theories of immunity, unless we may accept the suggestion, put forward in the footnote on p. 42, that real differences in strains of pneumococci do occur, and are of pathological significance.

The distribution given in Table XIV. is shown graphically in Chart IX. with its theoretical curve. This curve is of the "J" type, and starts at 0·45 of a day after the termniation of the pyrexial period of the first attack and ends at 36·03 days. It is well known that the probable error of the start and end of these "J" shaped curves is large, and further, that no satisfactory correction for the crude moments has as yet been described; we must not, therefore, lay too much stress on the fact that the theoretical curve terminates as shown. This fact, however, may suggest that second attacks are not all similar in character, and that those occurring after, say, the first month, owe their origin to a different set of causes. Moreover, there is in our previous figures (Table XIII.) a suggestion that the rate may fall in the second month after attack and rise again in the third, but owing to the smallness of the numbers and to other sources of error it will not be wise to do more than note these irregularities. Thus while not affirming that there is any demonstrable difference in the earlier and later distributions of second attacks, we must keep an open mind on the question until fuller data are available for investigation.*

*The medical officers of the Crown Mines have very kindly obtained for me the subsequent history of all cases of pneumonia occurring amongst *all* Natives employed on these mines for a period of one year, and the number of second attacks. These returns enable us to calculate the population at risk for each month for a twelve months table; but only tables corresponding to those of Table XIII. are here reproduced. Two cases of second attacks occurred in the portion of the table not reproduced, and the monthly attack-rate for second attacks for the twelve months table is 7·5 per thousand. It should not be overlooked that these figures refer to all Natives—Union Natives, East Coast Natives, and "Tropical Natives," and are not confined to the latter group as are those of Table XIII. The

primary attack-rate for pneumonia is much lower among the former groups than among "Tropical Natives." The total monthly attack-rate for the year 1912-1913 was approximately 3·80 per thousand.

Tables for "second attacks" from Crown Mines data:—

A. Population, i.e., number of "boys" exposed to risk of second attack.

Month after arrival in which 1st attack occurred.	Months after termination of 1st attack.						
	1	2	3	4	5	6	
1st	106	95	85	75	65	47	473
2nd	93	83	74	68	61		379
3rd	55	47	43	36			181
4th	52	46	40				138
5th	35	32					67
6th	31						31
	372	303	242	179	126	47	1269

B. Number of second attacks.

Month after arrival in which 1st attack occurred.	Months after termination of 1st attack.						
	1	2	3	4	5	6	
1st	0	1	1	0	0	0	2
2nd	2	1	0	1	2		6
3rd	1	0	1	0			2
4th	0	2	2				4
5th	0	0					0
6th	0						0
	3	4	4	1	2	0	14

C. Rates per thousand.

Month after arrival in which 1st attack occurred.	Months after termination of 1st attack.						
	1	2	3	4	5	6	
1st	0	11	12	0	0	0	4
2nd	22	12	0	15	33		16
3rd	18	0	23	0			11
4th	0	44	50				29
5th	0	0					0
6th	0						0
	8	13	17	6	17	0	11

Reference has already been made to the case mortality among primary attacks, and we have seen (Table II.) that the average rate for all groups of "Tropical Natives" attacked in the W.N.L.A. Compound is 39·2 per cent. The European case mortality for this disease is given in the text books as varying from 5 to 50 per cent. and over, for persons between 20 to 40 years of age. The European hospital case mortality is recorded as being from 20 to 40 per cent., but is on the average, higher than that obtained from the returns of private practitioners. Whether this difference is a result of hospital treatment, or is due to the selected character of the hospital population, it is impossible to judge, as I have been unable to find any records of the case mortality for patients who might be fairly considered comparable to those usually found in hospital, but who have, notwithstanding, been treated outside. We have already seen that there is probably no appreciable correlation between variations in the attack-rate and the case mortality, whether the increased attack-rate be "tribal" or "seasonal" in origin. The case mortality does not show any reduction with an increase in the length of residence although the attack-rate rapidly falls. Table XV. gives the rates for five-day periods.

TABLE XV.

Days after arrival on the Rand before admission to hospital.

Day after arrival ...	0-4	5-9	10-14	15-19	20-24	25-29	30-34	35-	Total
Cases	525	388	314	275	157	55	37	58	1809
Deaths	186	155	140	100	59	31	13	25	709
Case mortality per cent.	35·9	39·8	44·6	36·4	37·6	56·4	35·1	43·2	39·2

We should not expect, therefore, to find that the case mortality from second attacks would be lower than that from first attacks on the ground that a second attack must occur at a later date.

Out of sixty cases of second attacks occurring over a period of six months in the group of 8,123 "boys" (the "8000 experiment") there were thirteen deaths, giving a case mortality of 21·7 per cent., as against a case mortality of 32·1 per cent. for first attacks in this group. Out of eighty second attacks occurring in the W.N.L.A. Compound Hospital twenty-six died, giving a case mortality of 32·5 per cent., as against 39·2 per cent. for first attacks occurring in the same group. Thus in both groups we see that there is an apparent reduction in the case mortality from second attacks; at any rate, when they occur within a comparatively short period after the first. It must be remembered that the numbers are small and the probable errors, therefore, large. Using the ordinary formula $m\bar{p} + \cdot 67449$

$\surd m\bar{p}\bar{q}$ (which considering the relative size of $\bar{p}$ and $\bar{q}$ is probably legitimate), we find the expected number of deaths in the first case to be $19\cdot5\pm2\cdot44$ as against the observed 13, and $31\cdot6\pm2\cdot94$ as against the observed 26 in the second. If we form a "four fold table," and calculate the value of χ^2 in each case we reach $P=\cdot4016$ and $P=\cdot7149$.* These values show that the results may not improbably have arisen owing to errors of small samples. This reduction in case mortality, if, however, it be significant, may perhaps be due to the fact that those individuals who are least able to resist the invasion are eliminated from the population by the first attack, and if this were so, any assumption in regard to an acquired resistance by virtue of a first attack would be unnecessary.

It may be pointed out that third, and even fourth, attacks may occur within short periods. Amongst eighty Natives who contracted second attacks and whose history is known for a period of six months, six had a third attack, and one of these a fourth.

In Table XVI., p. 36, an analysis of the macroscopic *post-mortem* appearances in 688 cases of pneumonia is given. The term pneumonia, here as elsewhere, is applied only to cases in which consolidation of the lung was present, and fatal cases of pneumococcal infections not accompanied by consolidation of the lung have been omitted, as for example pneumococcal meningitis unaccompanied by pneumonia.

Meningitis was a complicating factor in 10·5 per cent. of the fatal pneumonias recorded in this table. In 85 per cent. of these cases the vertex of the cerebrum was affected, and this accords with the experience of other observers that this site is commonly involved in meningitis of pneumococcal origin. The number of these cases of pneumonia complicated with meningitis gives a much higher rate than those recorded by Musser & Norris,[6] or by Aufrecht.[5] In our series the cranial cavity was examined in every instance, and we are not informed whether this precaution was always taken by the observers quoted by these authors. The insidious onset of this complication and the uncertain character of the symptoms often makes the diagnosis of pneumococcal meningitis during life one of great difficulty, and in practice it is not rare to find exudation on the meninges in cases in which meningitis had not been suspected during life. The absence of symptoms may be due to the fact that not infrequently the vertex is the only region affected. Musser & Norris write:—"Among 49,028 collected clinical cases meningitis occurred in 206 (0·24 per cent. (sic)), and at autopsy in 180 out of 4,833 (3·5 per cent. (sic)). The percentages are actually 0·42 per cent. and 3·7 per cent. respectively, if the numbers stated are correct. Aufrecht writes:—

* See footnote to p. 52.

MORBID ANATOMY.

TABLE XVI.

Analysis of macroscopic leasions found on post-mortem examination of 688 fatal cases of pneumonia amongst "Tropical Natives" dying in the W.N.L.A. Compound Hospital on the Rand.

Organ affected.		Number of Cases.	Percentage in sub Group.	Percentage of Total Cases.	
Meningitis.	Vertex	25	34·7	3·63	
	Base	7	9·7	1·02	
	Ventricles	2	2·8	0·29	
	Vertex and Ventricles ...	2	2·8	0·29	
	Base and Ventricles... ...	2	2·8	0·29	
	Base and Vertex	17	23·6	2·47	
	Base and Vent. and Vert. ...	17	23·6	2·47	
	Total	72	100·0		10·47
Pericarditis.	Purulent	25	37·3	3·63	
	Serous	42	62·7	6·11*	
	Total	67	100·0		9·74
Recent Pleurisy.	Right Chest	92	33·1	13·37	
	Left Chest	63	22·7	9·16	
	Both Chests	123	44·2	17·88	
	Total	278	100·0		40·41
Empyema		19			2·76
Right Lung.	Upper Lobe	51	17·6	7·41	
	Middle Lobe	6	2·1	0·87	
	Lower Lobe	65	22·5	9·45	
	Upper and Middle	16	5·5	2·33	
	Middle and Lower	16	5·5	2;33	
	Upper and Lower	37	12·8	5·38	
	Three Lobes	98	33·9	14·24	
	Total	289	100·0		42·01
Left Lung.	Upper Lobe	14	8·8	2·03	
	Lower Lobe	77	48·4	11·19	
	Both Lobes	68	42·8	9·88	
	Total	159	100·0		23·11
Both Lungs.	Both Bases	83	39·5	12·06	
	Both Apices	6	2·9	0·87	
	Two Lobes	21	10·0	3·05	
	Three Lobes	54	25·7	7·85	
	Four Lobes	37	17·6	5·38	
	Five Lobes	9	4·3	1·31	
	Total	210	100·0		30·52
Lung not stated		30			4·36
Total		688			100·0

*This group may include some cases of serous transudation without pericardial inflammation.

"The frequent occurrence of meningitis in the course of pneumonia has been emphasised by Immermann & Hiller on the basis of earlier reports in literature. . . . . I myself in 253 cases, in almost all of which an autopsy was performed, found meningitis but seven times: besides this, I have noted meningitis three times as a complication of pneumonia in patients who recovered. According to this, among my 1,501 cases of pneumonia there were but ten cases of meningitis." These numbers are equivalent to rates of 0·67 per cent. of the total cases, and 2·8 per cent. of the cases in which a post-mortem examination was made.

Pericarditis has been recorded in 9·74 per cent. of the pneumonia autopses here analysed. Roughly speaking, one-third of them were instances of purulent pericarditis. As noted in the table, a certain number of cases classified as serous pericarditis may not, strictly speaking, be properly included under this heading, as the pericardium itself was not obviously inflamed, although the pericardial sac was distended with fluid. Musser & Norris[6] state, that acute pericarditis was observed 268 times in 2,128 autopsies (12·6 per cent.). In 38 cases complicating pneumonia, recorded by G. A. Scott,[6] the exudate was sero-fibrinous in 52·6 per cent. and purulent in 44·7 per cent. In six out of twenty-two cases recorded by Musser & Norris the pericarditis was fibrino-purulent, a percentage of 27·2. The figures of both Scott and the latter authors are small; and the value of the percentage of purulent cases in our returns lies between the values obtained from their figures; we cannot say, therefore, that this form of pericarditis is either more or less common among these Natives than among Europeans. As regards the total number of cases of pneumonia complicated by pericarditis irrespective of the type it would seem probable that pericarditis is rather less common among "Tropical Natives" than among Europeans.

Pleurisy. It is perhaps doubtful whether this condition should be considered as a complication of pneumonia. Musser & Norris write:— "Pleuritis occurs in practically all the cases, or at least in those cases in which the inflammatory process reaches the surface of the lung." Amongst autopsy records from 974 cases they record pleural effusions as occurring in 41·58 per cent., which corresponds closely with the percentage found in Table XVI. It is worthy of note that the pleural effusion with adhesions are sometimes found on the opposite side of the chest to that containing the consolidated lung.

Consolidation of the Lung. In regard to the lobe affected the following distributions may be compared with that recorded in Table XVI.

TABLE XVII.

Lobe affected.	Aufrecht per cent.	Norris per cent.	Musser & Norris. per cent.	Table XVI. per cent. ‡
Right Upper ...	9·6	11·0	11·1	7·75
,, Middle ...	2·3	2·0* (3·0)	2·2	0·91
,, Lower ...	22·6	29·0	27·8	9·88
,, Two lobes ...	7·5		6·3	10·49
,, Three lobes ...	10·0	9·0	8·2	14·89
,, Total ...	52·0	51·0* (52·0)	55·6	43·92
Left Upper ...	3·9	4·0	4·5	2·13
,, Lower ...	24·8	23·0	24·6	11·70
,, Both ...	6·7	7·0	7·6	10·33
,, Total ...	35·4	34·0	36·7	24·16
Both Lungs ...	12·6	21·0* (13·0)	7·5	31·91
Number of cases ...	1501	493	4062	658

* Percentages areas given in the original, figures in brackets are rates calculated from the actual figures recorded.

‡ Rates given here are not identical with those of Table XVI., as the 30 cases in which the lung affected was not specified have been omitted.

Adami,[8] quoting Orth, states that the right lung is affected in fifty-two per cent. of cases, the left in thirty-three per cent., and both lungs in fifteen per cent. of cases. In the figures from the Native cases we find both lungs are much more commonly affected than in any of the examples quoted, and also that the right lung is rather more frequently attacked than is the case among European patients. Further, among the Natives the disease is not so commonly limited to one lobe: this may indicate that the Native is less resistant to the extension of the infective process than the European.

The appearance of the lung as seen at the necropsy in regard to the " stage of hepatization " does not bear a constant relationship with the length of illness before death. In Table XVIII. the stage of the portion of the lung in the most advanced condition is recorded.

This table illustrates the fact that the " stages of hepatization " of the lung are very variable in the time of their appearance. The lung may be found in the stage of " grey hepatization " within a day or two of the apparent onset of the symptoms, or may still be in the " red stage " after ten or more days of illness. In the earlier part of the table there are no doubt a certain number of cases where the date of admission does not correspond even approximately to the com-

TABLE XVIII.

Appearance of the Lung at the Necropsy.

Day of death after admission.	Acute congestion. Commencing consolidation.	HEPATIZATION.				Resolving.	Normal.	Total.
		Early Red.	Red.	Red to Grey.	Grey.			
1— 5	12	21	38	47	65	5		188
6—10	1	10	44	57	77	7		196
11—15			9	18	39	12		78
16—20		3	3	2	16	4		28
21—25			2	4	10	1	1	18
26—30					7	5	4	16
31—40		1	1	1	6	2		11
41—50				2	7	1	1	11
51—60					1		1	2
61—70						1	1	2
71—80						1		1
	13	35	97	131	228	39	8	551
Percentages	2·4	6·4	17·6	23·8	41·4	7·1	1·4	

TABLE XIX.

Day after admission to hospital on which death occurred.

Day after admission.	No. of deaths.	Day after admission.	No. of deaths.	Day after admission.	No. of deaths.	Day after admission.	No. of deaths.
1	29	8	56	15	16	46— 50	5
2	32	9	39	16—20	38	51— 55	1
3	52	10	27	21—25	24	56— 60	1
4	63	11	27	26—30	21	61— 65	1
5	60	12	27	31—35	14	66— 70	2
6	54	13	23	36—40	6	71— 80	1
7	62	14	16	41—45	10	81— 90	0
						91—100	2
						Total	709

mencement of the illness, and in the latter part it may be an attack in a second lobe which has been noted in the post-mortem report, when the first lobe to be affected may have become normal in appearance. Even when reasonable allowance has been made for these sources of error we still see that the rate of change in the lung tissue, as judged by the general appearance, must vary within wide limits; and thus, in any individual case, the stage of hepatization is but little guide to the length of illness before death.

Chart X. represents the number of deaths occurring on each day after admission to hospital. The numbers are given in Table XIX.

~~table:—~~

This distribution is almost certainly heterogeneous, and cannot, I find, be fitted by any single "Pearson curve." If we eliminate a portion of the tail as shown on the chart by the dotted line, an approximation to the bulk of the distribution may be obtained; giving the mean period before death as 7·5 days, and the modal period as three days. Further adjustments have been attempted without any very marked improvement in fit, and it would appear almost impossible unless unreasonable alterations are made, to increase the position of the mode to more than about 3·8 days. An explanation of the want of fit of the original distribution may be that the later deaths are not due, strictly speaking, to pneumonia, but rather to some super-added infection, for example by a streptococcus, and if this had not occurred the pneumonia would not have been fatal. If this be the explanation we could obtain an approximate measure of the number of deaths due to intercurrent or subsequent infections. Further study may lead to a more satisfactory analysis of this distribution, but there is little doubt that it is heterogeneous in character and does not represent deaths due solely to pneumococcal infections.

SECTION III.

A Consideration as to whether there be any Evidence that Pneumonia is an Infectious Disease.

Before any discussion of the question whether pneumonia may be spread from case to case can be usefully undertaken it is necessary to consider the meaning of the terms " Infectious " and " Infective." Considerable confusion seems to exist at present in medical circles as to what these terms respectively connote.

If we turn to the definition of *Infectious* in the New Oxford English Dictionary we find the following:—

" 1. Having the quality or power of communicating disease by infection; infecting with disease; pestilential; unhealthy."

" 2. Of diseases: Apt to be communicated or received by infection; liable to be transmitted from one person to another by means of air or water."

Under *Infective* we find:—

" 1. Having the quality of affecting injuriously or tainting." . . .

" This is the old and almost obsolete use."

" 2. Having the quality of infecting with disease, or spreading disease by infection; infectious." . . . . .

" Modern use."

It would appear from these definitions that the modern use of Infective is as a synonym for Infectious.

In Fagge & Pye Smith[10] we find the following statements:— " From an early period it has been observed that certain diseases are contagious or ' catching,' either by direct contact with the sick person, or by means of clothing or buildings, or by conveyance through the air of a presumed *materies morbi:* When actual contact was necessary to reproduce it the disease was called *contagious:* when it was transmissible by the air it was given the wider name of *infectious*. . . . The distinction is not fundamental."

" The word *infective* has been used of late to denote processes which spread, not always from one person to another, but from one tissue or organ of the body to another."

Here we have a definition of infective which is not synonymous with infectious, as some infective diseases are not infectious.

Notter & Firth[11] write as follows:—

" This conception of the origin of the causes of disease if interpreted literally, implies the belief that every single case of each infective disease is the offspring or result of an antecedent case of the same disease. A little reflection and experience soon indicate that

the doctrine is too inelastic for general acceptation, because certain of these diseases affect the lower animals as well as man, and consequently the antecedent case of a given instance of such disease need not be a human case; while as regards the others, which are known to affect man only, the pre-existing case must be sought in man alone." From the same authors, under the heading "The Infective Diseases," we find the following:—"According to the manner in which these diseases are transmissible from one person to another, so are they spoken of as being either infectious, contagious, or inoculable."

Here again we have infectious diseases regarded as a sub-group of infective diseases, but in the first quotation from the same authors we are told that the source is not always a human case of the disease, and we must therefore include diseases derived from animals. Are we then to exclude from the term infective diseases cases of bacterial infection which arise, not from any previous case, but from an organism which was previously a saprophyte,* for example some members of the coli group? Or again, as in the case of tetanus, where the invading organism is not derived from a previous case of the disease, or at least where such connection cannot be traced.

We find that Sir W. Osler[7] classifies under the heading "Specific Infectious Diseases" the following sub-groups:—

A. Bacterial Diseases.

* "Parasite" would be possibly a better term to use in this connection than "Saprophyte." It almost seems as if a new term were wanted to describe an organism which can exist and multiply on the surfaces of its host without giving rise to any abnormal symptoms, but which can become pathogenic under altered conditions. Whether this change from "harmless parasite" to "pathogenic parasite" be due to alterations in the host, or to alterations in the type of the organism, or to both factors together, is a matter of conjecture. I believe, the most likely hypothesis is, that an alteration in type occurs which is usually occasioned by some antecedent alteration in the fluids or tissues of the host. That members of the coli group may take on pathogenic characters is established, and it is possibly true also of members of other groups; for instance, the diptheroid group, and the tubercle or "acid fast" group. It also seems to me probable that a "pneumonia" group exists, and that we shall come to recognise the different strains of pneumococci as being as separate in character as the members of the coli group, and perhaps as even more easily altered in character. Dr. F. S. Lister's recent work in this Institute, which will shortly be published, appears to support this view, and if confirmed will place on a much more solid basis what is at present merely a speculation. The presence of the pneumococcus in the throats of the apparently healthy, its association with epidemic outbreaks of acute naso-pharygeal catarrh, its causal relationship with lobar pneumonia, the marked differences between various strains in their effects on experimental animals, and the rapid alterations which often occur under laboratory conditions—all these and other facts point to the possibility or even probability that the organism known as Fraenkel's pneumococcus is not a single entity but rather a group of pneumococcal organisms. This question is referred to here because it has a bearing on the problem of infection.

B. Fungus Infections, not bacterial.
C. Protozoan Diseases.
D. Diseases due to Metazoan Parasites.
E. Infective diseases of doubtful or unknown etiology

Under "A" we find tetanus, and it is therefore classed as an infectious disease, and in the sub-group infective diseases we find smallpox. The term "infectious disease" then apparently corresponds to Notter & Firth's "infective disease," and is in disagreement with the definition given by Fagge & Pye Smith. But on page 78.7 we find this sentence:—"Clinically, the *infectious nature* of pneumonia was recognised long before we knew anything of the pneumococcus." And further:—"As many as ten occupants of one house have been attacked." This latter remark, in its relation to pneumonia, will be discussed later on. Thus "clinically infectious" has clearly a different meaning to "infectious." If we start by defining all bacterial diseases as infectious, then having once shown that a disease is causally connected with a bacterium it is unnecessary to discuss whether or no it be infectious.

This classification has, to my mind, several objections; to make the word "*infectious*" practically synonymous with "*due to a micro-organism*" in respect to diseases, does not help us to a more accurate method of thought, and has, moreover, the serious disadvantage that it changes the ordinary, or at least the older, meaning of the word. This change has led, and will lead to constant misunderstanding and confusion, and the necessity of introducing the expression "clinically infectious" exemplifies the author's need for a new term to correspond with the old usage of the word infectious with its meaning of "catching."

Let us turn now to definitions given in text-books of Infectious Diseases, for the term when thus employed has an old and well established meaning, totally different, however, from that used by Sir W. Osler. Ker,[12] in his admirable text-book, defines infection thus:—"By infection is understood the invasion of the tissues by a pathogenic bacterium. . . . And here it may be remarked that it is difficult to draw a hard and fast line between pathogenic and saprophytic bacteria." . . . "It is, with our present knowledge of the bacterial causes of infectious diseases, difficult to draw fine distinctions between contagion and infection. . . . A pathogenic organism, however, may be regarded as one capable of producing disease in man under natural conditions." Here we are left wondering what the phrase "natural conditions" may imply, and whether we are to exclude from the classification "pathogenic bacteria" those which produce disease in animals but not in man.

The following definition is given by Goodall & Washbourn[13]:— "Diseases which are transmissible from one individual to another are said to be *contagious* or infectious. Strictly speaking, the term infectious is applied to diseases transmissible through the air, while the term contagious is used of diseases which are spread by direct contact, or by infected articles." In this definition we have returned to a definition more like that given by Fagge & Pye Smith, and more in accordance with the general or "lay" use of the word.

It is surely meaningless to speak of one disease as more or less infectious than another, or to use such an expression as "highly infectious" for a disease like small pox, if we are going to use the word as synonymous with bacterial, in Sir W. Osler's terminology; but there is clearly a need for some term corresponding to the term infectious as employed and defined by Goodall & Washbourn.

Historically, it is not difficult to see how the extension in the use of the term may have taken place; but whether such an extension is scientific, that is whether it helps us to classify the facts more accurately, is perhaps open to doubt. Some argument such as the following may have been used—"catching diseases" were in some instances proved to be due to micro-organisms, therefore, by analogy all such diseases are probably dependent on some micro-organism. The micro-organisms of disease are specific, and therefore the organisms in one case must have been obtained directly from those of a previous case. This must be true therefore of all diseases due to micro-organisms; and thus all such diseases are infectious, and we should, logically, extend the term "infectious diseases" to the whole group of complaints due to micro-organisms, and even further, to those of metazoal origin.

If this were the argument employed it is clearly unsound. A series of assumptions are made which have not been proved; they are, however, assumed to be true, and a generalisation drawn which has had the effect of leading the student to regard the question as settled This tends to obscure the need for a patient search for the evidence which shall prove or disprove them. However useful these hypotheses may be in their legitimate sphere, they are dangerous when interpreted as reliable theories; and moreover, grave doubt has already been thrown on the validity of some of them. Saprophytic organisms have been shown to acquire pathogenic properties, and thus an antecedent case of the disease need not necessarily be postulated.* The specific

* If for the sake of argument we assume that healthy people communicate the pneumoecoccus to others who also remain healthy, so that a large number of the population harbour the organism as a harmless parasite, it would serve no useful purpose from a practical point of view to class pneumonia as an infectious disease; unless indeed the organisms spread by the sick had different properties, i.e., were virulent to the healthy, in which case evidence of this should be found in the statistics.

character of several pathogenic bacteria is also seriously in doubt. That a very large number of pathogenic bacteria may live as saprophytes is proved by the varieties which can be grown on artificial media, and although it may be reasonable to assume that similar conditions are not paralleled in Nature this has not been established as a fact.

All these questions require careful investigation with an open mind, and any phraseology which implies that they have been proved is, it seems to me, retrograde. If this be admitted, the need for a word which will express the common meaning "catching" and will also connote a definite train of events formerly covered by the word infectious, is evident.

Considering the very old established use of this word it would not seem wise to abandon it and further complicate the terminology by the introduction of another, despite the fact that one of the leading text-books of Medicine has extended the meaning as described above.

It may be considered by some that the term "communicable" would be suitable, seeing that there is a growing tendency to use this word in place of infectious. It seems to me that this word, used in the sense "infectious," is not free from ambiguity. It suggests that the disease is capable of being communicated, rather than of communicating itself: that is to say, a suggestion of a deliberative agent is introduced. Thus, Vaccinia in man is a communicable disease, but scarcely infectious in the sense in which it will be defined here. Again, the term "catching" might be used as an alternative: but it is also liable to misuse, and does not seem suited to accurate thought: for we constantly hear such expressions as "catch a chill" or "catch a cold" where the suggestion of contact with a previous sufferer is not intended. On the whole, then, it would appear to me better to retain the term "infectious" and try to define it so as to make its use free from ambiguity.

The following definition of an infectious disease is therefore suggested:—*An "infectious disease" is one which may be spread from the affected to the healthy, and is such that the healthy person incurs a risk of contracting the disease by associating with a person suffering from it, either directly, or indirectly through the medium of clothing, furniture, food-stuffs, etc., which have been contaminated by the sick.* It may be objected that a disease spread by healthy persons, that is by those who have never suffered or exhibited any symptoms of illness, should nevertheless be classed as infectious. Such a disease would only be excluded from the definition infectious if those actually showing symptoms did not spread it, or did not constitute any increased risk to the population; that is to say, that those in their immediate neighbourhood did not, on the average,

suffer more than people at a distance. From the practical point of view, it seems improbable that any administrative steps could be taken to deal with a disease which spreads as commonly from the healthy as from the sick; but, if acquired more commonly from the sick, it will fall within the limits of our present definition, and so statistical evidence should be forthcoming in proof of such a fact.

It will be seen, therefore, that the proposed definition is purely utilitarian, and if accepted we have a right to ask for proof that association of the healthy with the sick constitutes a risk to the former, before we consent to classify any disease as infectious.

In regard to diseases spread through animals, three classes must be considered:—

Firstly. Diseases in which the animal is itself affected, and in the spread of which man *need* play no part; e.g., Tuberculosis or Glanders.

Secondly. Diseases in which the animal (usually an insect) is necessarily the intermediate host, e.g., Malaria or Tick Fever.

Thirdly. Where the animal concerned (again usually an insect) is merely a mechanical carrier of infection, e.g., Bubonic Plague.

The first group will fall within the terms of the definition, if, when spread to man he in turn infects his neighbours. Possibly a special category must be made for a few parasitic diseases where man and an animal are alternate hosts. The third group will necessarily be classified as infectious because (insect) carriers will be more numerous in the immediate neighbourhood of the diseased. In regard to the second group, and to a less extent the third also, a problem arises of considerable practical importance. It will be evident that when an special animal host is essential to the spread, that the disease will only become infectious when the intermediate host is present. Thus a disease may be infectious in certain regions and cease to be infectious when imported into others; or again, it may be infectious only at certain seasons. This is a distinction of some practical importance.

Further, it is conceivable that a disease might be infectious among one race and not among another; habits and customs might be determining factors in its spread, or constitutional immunity might exist. For instance, a race may be conceived as having developed through selection an immunity, and the introduction of a case, therefore, into such a community would not be a source of danger. In such circumstances this disease could not reasonably be classified as infectious in respect to such a population.

It is evident, then, that this definition is purely a practical one, but from an administrative point of view is, it appears to me, of importance; and further, it is, I think, a reversion to the older and more commonly accepted use of the word. In the following para

graphs the word "infectious" will be used with the meaning given to it in the above definition.

Many authors, from v. Jürgensen in 1874 to those of the present day, have claimed that pneumonia is an infectious disease, not in Sir W. Osler's sense that it is due to a micro-organism, but apparently in the sense in which the word has just been defined. Musser and Norris[6] write as follows:—"To sum up, we are forced to the conclusion that pneumonia is a transmissible disease, to be classified with the infectious fevers, and like them to be guarded against by the application of antiseptic principles." They subsequently proceed to describe the pneumococcus of Fraenkel as *the micro-organism of lobar pneumonia*, and thus under this term they are dealing apparently with a specific disease, and not merely with a group of signs and symptoms. Why then, we may ask, do they include the "Middlesborough Epidemic" among the list of examples quoted to indicate that pneumonia may be spread by infection, when the organism described by the investigators of this outbreak was not the pneumococcus? No evidence in favour of the spread by infection from case to case can be considered valid if the identity of the associated organism be not determined. Further, they state that in Kimberley this disease is limited to the Kaffirs: how such a curious misstatement could have arisen it is difficult to understand, but in any case evidence must surely be much more carefully sifted, than appears to have been done by these authors, before valid conclusions can be drawn.

The "Middlesborough Epidemic," so often quoted in support of the contention that pneumonia is an infectious disease—and amongst others by Notter & Firth[11]—shows that outbreaks of a disease similar to, but not identical with, pneumonia may occur; we must, therefore, be particularly cautious before concluding that an epidemic is due to the pneumococcus in the absence of bacteriological proof. In most of the instances quoted by the authors of text-books no mention of the organism associated with the disease in question is made, and there is therefore no direct evidence that the disease was due to the pneumococcus of Fraenkel, which organism we are assuming to be the cause of Pneumonia. This is a criticism which must apply to all evidence collected prior to the recognition of this diplococcus; and it also applies, so far as I know, to the instance of ten cases in the same house, already quoted from Sir W. Osler's book. This, however, is not the only exception which must be taken to this example when adduced as a proof of infection.

To prove the infectious nature of any disease it is not necessary merely to collect examples of runs of cases in one house or family; we must show that such runs occur more frequently than they should do on the laws of chance, supposing every similar individual in the

community equally liable to contract the disease, whether he be, or be not in contact with one already affected. So far as I know, no attempt up to the present has been made to follow such a method in regard to pneumonia.

Our figures deal entirely with the African Native from "tropical areas," and any conclusions which we draw from them in regard to the problem of infection must not be applied unreservedly to Europeans in South Africa, much less to Europeans in Europe. A careful statistical analysis of European data, is, I believe, the only way in which these problems with regard to the latter groups can be satisfactorily settled, and the argument from analogy, if used at all, must be employed with extreme caution.

In the "8000 experiment," records of the medical history of all the Natives included were kept in each case for six months, and in some for longer periods. All these Natives passed through the Witwatersrand Native Labour Association's Compound, where they were given serial numbers. On arrival in the compound the "boys" line themselves up under Captains, and those coming from the same district stand together. As a consequence relations, friends, and acquaintances will, on the whole, tend to carry consecutive numbers. On allotment a group under any Captain is not broken, so that subsequently on the mine "boys" carrying consecutive numbers are in closer association than those with more distant numbers. In Portuguese territory a similar method of numbering is adopted, and it appears that the order in which the "boys" obtain their numbers, although not identical with the W.N.L.A. order, is much the same; in practice one finds little difference in the results calculated from either the Portuguese or W.N.L.A. Depôt Numbers.

If we suppose that one case of pneumonia in a community of Natives does not increase the risk to the others, we may expect that the various cases of this disease will not be unduly grouped. Grouping might, nevertheless, occur owing to a variation in the prevalence of the disease on different mines; a point which will be discussed later. It will be noted that runs of twos, threes, and so on, are to be expected even on the assumption that the "laws of chance" alone determine the distribution. By runs or sequences, we mean affected Natives carrying consecutive numbers. If, however, the disease tends to spread from the sick to the healthy, that is, it is infectious, then we should expect that runs of twos, threes, etc., will be more common than if chance were the only factor concerned.

By means of the following formula we can calculate the most probable chance distribution for the appearance of sequences.

Let N = number of "boys" in the series,

m = number not attacked,
n = number attacked,

then it can be shown that the various terms of the following expression will represent the most probable distribution for the number of sequences,*

$$\frac{m\,(m-1)}{N-1}\left[1+\frac{n}{(N-2)}+\frac{n\,(n-1)}{(N-2)\,(N-3)}+\frac{n(n-1)\,(n-2)}{(N-2)(N-3)(N-4)}+\cdots\right]$$

The successive terms can be easily calculated from the preceding one; thus, if R_0, R_1, R_2 etc., represent the terms, and if

$$R_0 = \frac{m\,(m-1)}{N-1}$$

$$R_1 = \frac{n}{N-2}\,R_0$$

$$R_2 = \frac{n-1}{N-3}\,R_1 \quad \text{etc.}$$

Now, even if the laws of chance alone are operative in determining the distribution, we must not expect that the experimental numbers will be identical with those of the most probable distribution; but we can, by Pearson's method of "Goodness of Fit,"[14] find the chance that the observed distribution has arisen owing to errors of random sampling.‡

Let us now turn to the actual results from 8,088 Natives ("8000 experiment"). Regarded as one group, we find:—

N = 8,088, m = 6,988, n = 1,100.

* The first term represents a zero sequence, i.e., where two unattacked "boys" come together, the second a sequence of one, i.e., where there is an attacked "boy" with an unattacked "boy" on each side, the third a sequence of two attacked between unattacked, and so on.

† I am indebted to Dr. Troup for most of the mathematical analysis in the proof of this problem, and although it is not yet free from difficulty, the matter is too technical for discussion here. We hope to publish it elsewhere shortly.

‡ The meaning of this will be made clear if we consider a simple example. If we toss ten pennies, the most probable result will be five heads and five tails; that is to say, if we repeat the experiment 1,000 times we shall find five heads and five tails to be more common than any other result. But we shall find that four heads and six tails, three heads and seven tails, and so on, also occur. If, therefore, on the first throw we find three heads and seven tails, we must not straightway assume that the coins are biased. If we knew nothing about our coins we should then proceed to calculate the probability of such an event occurring on the assumption that they were not biased, and our result would be in the form $P=x$ where x is the probability that such a divergence would occur on the laws of chance. Thus if the probability is $P=0{\cdot}2$ we can say such a divergence from the most likely will, on the average, arise twice in every ten such examples.

TABLE XX.

Sequences of	Theoretical number.	Observed number.
1	821	768
2	112	122
3	15	16
4	2	8
5, 6	0·31	1

P=0·00004

Here we have a defect in sequences of one and an excess over theory at the higher groups; and considering the value of "P" we may conclude that it is highly unlikely that a distribution such as this has arisen owing to errors of random sampling. It is possible, however, that the heterogeneous nature of this population may be responsible in part for the value found. This group has, therefore, been sub-divided according to the territorial areas from which the Natives were recruited.

TABLE XXI.

Mozambique Natives.

Groups.	Theory.	Observation.		
1	231·86	214	N=2304	
2	31·03	34	m=1995	
3	4·15	5	n= 309	
4	0·66	3		P=0·017

Here only seventeen times out of 1,000 could such a divergence from theory be expected on the laws of chance.

TABLE XXII.

Angoche or Parapat Natives.

Groups.	Theory.	Observation.	
1	98·11	85	N= 889
2	15·06	15	m= 752
3	2·30	3	n= 137
4	·38	1	
5		0	2
6		1	

P=0·020

Here only twice in a hundred trials is a fit as bad as this to be expected.

TABLE XXIII.

Quilimanes.

Groups.	Theory.	Observation.		
1	121·05	128	N=1141	
2	17·45	13	m= 976	
3	2·50	1	n= 165	
4	·39	2		P=0·028

TABLE XXIV.

Portuguese Nyassas.

Groups.	Theory.	Observation.		
1	232·81	210	N=2171	
2	34·26	42	m=1851	
3	5·03	6	n= 320	
4	0·88	2		P=0·148

In this case, as in that of the Mozambiques, the ones are in defect, the twos, threes, and fours in excess.

TABLE XXV.

Tete & P.I.T.

Groups.	Theory.	Observation.		
1	140·13	139	N=1572	
2	15·81	18	m=1394	
3	1·77	1	n= 178	
4	0·20	0		P=0·842

Here we could not expect a better agreement between observation and theory on the laws of chance.

Taken together, these tables show in a high degree, the improbability that the observed deviations from theory have arisen by chance; but we must not necessarily assume that the deviations are therefore due to infection.

Considering the conditions under which the Natives live and work on the mines, the chance that an affected "boy" will spread the disease, if it be infectious, to a "boy" carrying a neighbouring number, will not be much less than the chance of his spreading it to a consecutive number, while the likelihood of his conveying it to a "boy" with a distant number is much more remote.

If then the disease be spread from case to case we might expect to find—if we reverse our problem and calculate the most probable distribution of the runs of unaffected "boys"—that the small runs, of say ones, twos, three, and also the very long runs, will be in excess

of theory, with the consequence that the runs of intermediate size would be in defect.

To obtain the theoretical distributions for the new series we must re-write our formula, thus:—

$$\frac{n\,(n-1)}{N-1}\left(1+\frac{m}{(N-2)}+\frac{m\,(m-1)}{(N-2)(N-3)}+\ldots\ldots\right)$$

Here the first term or the sequences of 0 is of importance. A sequence of 0 now means two affected "boys" with no unaffected "boys" between. Thus a run of three affected "boys" is equivalent to two sequences of 0, and so on. The value of the probability for the whole group of 8,088 Natives is $P=0\cdot002$ approx. The 0's are considerably in excess and supply 8·19 to the value of χ^2*14, the 1's supply 0·94, and the 3's 1·04, the 2's are slightly in defect, 7's, 8's and 10's are considerably in defect, contributing together 12·63 to the value of χ^2. Excesses are again found at the tail of the distribution.

For the Mozambiques (23 groups), $P=0\cdot33$ approx. The χ^2 value was not calculated for four runs at the tail, namely, one each of 43, 46, 53 and 71; these will decrease the value of P, but the labour of carrying the calculation to 71 terms would be hardly justified by the increased accuracy to be obtained. Here runs of 0's and 1's are in excess, but otherwise no very marked abnormality in the distribution occurs. After 25 the tail shows errors in excess.

The same general statement is true of the Angoche Natives, but marked divergence from theory occurs in the 0's, which group contributes 4·77, and also in the groups 18 to 20, which together contribute 7·81 to the χ^2 value, $P=0\cdot07$.

Table XXVI., page 53, is given as an example of the method. Differences are calculated only to nearest whole number in the column headed "Theory."

A similar table worked from the Portuguese numbers gave $P=0\cdot65$.

The distribution for the Nyassa's leads to, $P<0\cdot02$. In this distribution there is a marked excess in the groups 0, 1, 2 and 5, and a marked defect in groups 7, 8, 9, 10 and 11; excesses again appear after 24.

Among the Tete & P.I.T. Natives excesses are found in the groups 8 to 14, and defects in 2, 4, 5, 6 and 7, and the tail of the distribution

*The meaning of this symbol will be found in the essay of Professor Pearson, already referred to.14 It is the sum of the differences of the theoretical and observed values squared and divided by the theoretical value. Thus if m = observed value, and m' = theoretical value, then $\chi^2_1 = S\left[\frac{(m-m')^2}{m'}\right]$.

TABLE XXVI.

Quilimane Group.

Group.	Theory.	Observation.	Obs.—Theory.	χ^2
0	23·74	21	−3	0·3791
1	20·34	24	+4	0·7866
2	17·43	11	−6	2·0654
3	14·93	15	0	
4	12·79	19	+6	2·8507
5	10·95	9	−2	0·3653
6	9·38	9	0	
7	8·03	10	+2	0·4981
8	6·87	5	+2	0·5822
9	5·88	6	0	
10	5·03	5	0	
11	4·30	6	+2	0·9302
12	3·68	5	+1	0·2717
13	3·15	3	0	
14	2·69	4	+1	0·3717
15	2·30	4	+2	1·7391
16 to 20	7·34	3	−4	2·1798
21 to 25	3·34	2	−1	0·2994
26 to 30	1·54	3	+1	0·6494
31 to 45	1·28	1	0	
				13·9687

P=0·78

is not markedly, if at all, in excess of theory. P=0·11 approx.

These results are summarised in Table XXVII.

TABLE XXVII.

Summary of results in Tables XXI. to XXVII.

Total.	Value of "P" for sequences of Pneumonia.	Value of "P" for sequences of unattacked Natives.
Total	0·00004	0·002
Mozambique ...	0·017	0·33
Angoche ...	0·032	0·07
Quilimanes ...	0·028	0·78
Nyassas	0·148	<0·02
Tete & P.I.T. ...	0·842	0·11

We thus reach the conclusion that if every "boy" within a group has an equal chance of contracting pneumonia—which of course is one

of the assumptions which must be made if the distribution is to be described by the laws of chance—then, with two or possibly three exceptions, we can assert that the observed divergencies are unlikely to have arisen by chance.

But infection from case to case is not the only cause which might bring about this result. It is quite conceivable that certain sub-groups within the territorial groups may be more susceptible than others. We know that in regard to race, altitude, and climate of their homes, no group is even approximately homogeneous. Before making any assumption as to the cause of the divergencies we must study the matter more in detail.

It may here be interesting to record that the values for " P " in the case of a Chicken Pox distribution amongst the Quilimane Natives (for the same group of Natives as in Table XXVI.) was found to be zero to more than six places of decimals; and that the χ^2 values for both tables, viz., that grouped according to sequences of cases of Chicken Pox and sequences of the unattacked, is larger in proportion to the number of sub-groups than any of the values for the pneumonia distributions. In the Chicken Pox distribution (the unattacked), two runs occur, one of 150 and the other of 445; these are very considerably in excess of any found in the pneumonia distributions.

The dates of admission to hospital of the Natives suffering from pneumonia who constitute the runs of threes, fours, and six, are given in Table XXVIII. and XXIX. The small numbers between the dates show the number of intervening days.

We notice on reference to the table of " sequences of threes " (TableXXVIII.) that on only two occasions were all the cases admitted to hospital within thirty days. Among the groups of four and over there are three runs of threes admitted within this period, and none of four and over. Further, we note that a considerable number of pairs occurred in these groups with very short intervals between the admissions; this, if we assume infection from case to case, would imply a very short incubation period—unless both cases were infected from some common source and not the one from the other—whereas in the runs of threes we must assume a much longer period. The incubation period of most infectious diseases varies within fairly narrow limits, in the absence, therefore, of definite information each observer will, for the present, have to decide for himself what range he considers it reasonable to assume as a working hypothesis in respect to lobar pneumonia.

To return to a subject previously mentioned, namely, the varying prevalence of pneumonia on different mines. It is quite possible that there may be some factor or factors operative to a different extent on

TABLE XXVIII.

Table giving dates of admission to hospital of Natives bearing consecutive numbers in sequences of three.

Mozambique.	Angoche.	Nyassa.	Quilimane.
28.10.12	18. 9.12	28. 8.12	14. 9.12
114	*71*	*14*	*74*
19. 2.13	28.11.12	11. 9.12	27.11.12
42	*5*	*48*	*96*
2. 4.13	3.12.12	29.10.12	3. 3.13
8. 9.12	30. 9.12	6.11.12	
59	*52*	*5*	
6.11.12	21.11.12	11.11.12	
40	*162*	*39*	
16.12.12	2. 5.13	20.12.12	
			Tete.
23.11.12	30.10.12	16. 8.12	13.11.12
31	*2*	*1*	*25*
24.12.12	1.11.12	17. 8.12	6.12.12
27	*138*	*150*	*39*
20. 1.13	19. 3.13	14. 1.13	14. 1.13
23.11.12		1. 2.13	
0		*10*	
23.11.12		11. 2.13	
136		*16*	
8. 4.13		27. 2.13	
22.11.12		16.10.12	
61		*55*	
22. 1.13		10.12.12	
14		*79*	
5. 2.13		27. 2.13	
		16.11.12	
		21	
		7.12.12	
		3	
		10.12.12	

TABLE XXIX.

Table giving dates of admission to hospital of Natives bearing consecutive numbers in sequences of four and over.

Mozambique.	Angoche.	Nyassa.	Quilimane.
22.11.12	16. 2.13	26. 8.12	23.10.12
0	*22*	*0*	*5*
22.11.12	10. 3.13	26. 8.12	28.10.12
1	*25*	*78*	*115*
23.11.12	4. 4.13	22.11.12	20. 2.13
69	*3*	*3*	*28*
31. 1.13	7. 4.13	25.11.12	20. 3.13
4.10.12	22.11.12	31.10.12	11.10.12
39	*35*	*27*	*30*
12.11.12	27.12.12	27.11.12	10.11.12
6	*65*	*1*	*42*
18.11.12	1. 3.13	28.11.12	22.12.12
17	*12*	*135*	*47*
5.12.12	13. 3.13	12. 4.13	7. 2.13
	14		
	27. 3.13		
	32		
	28. 4.13		
16. 9.12			
126			
20. 1.13			
70			
31. 3.13			
24			
24. 4.13			

one property as compared with another; this would cause an undue grouping of cases when they are examined by the methods just adopted. An estimate of the effect of this factor may be arrived at in the following manner. We may regard each series of twenty-five "boys" as a group by itself, and determine how many cases of pneumonia occurred in each group. We can then calculate how many of these groups should have had no cases of pneumonia, how many one case, and so on, by the formula[20]:—

$$p_s = \frac{m\,(m-1)^{n-s}\,n!}{s!\,(n-s)!\,m^n}$$

TABLE XXX.

Number of cases of pneumonia occurring in groups of 25 Natives with consecutive numbers.

Number of Cases.	Observed frequency of occurrence.	Theoretical frequency.	Difference.
0	16	10	+ 6
1	44	36	+ 8
2	55	61	— 6
3	58	70	—12
4	69	60	+ 9
5	30	41	—11
6	23	24	— 1
7	14	11	+ 3
8	7	5	+ 2
9	1	2	— 1
10	2	1	+ 1
11	2	0	+ 2
	321	321	

It will be noticed that while considerable differences are found between the observed and the expected frequencies, the plus and minus differences alternate, no definite order being maintained.

If infection from case to case were the cause of the departure of the observed from the theoretical frequency, we should expect the groups with the larger numbers of affected to be considerably in excess, while those with the smaller numbers should be uniformly in defect: this, however, is not so. This method of dealing with the problem is not entirely free from objection, nevertheless it should, it seems to me, be sufficiently accurate to demonstrate any general law underlying the grouping should such exist.

Two other sources of error also exist; firstly, all the Natives comprised in the foregoing tables were not under observation for the same length of time; and secondly, the attack-rate is a function of the period of residence on the Reef. Allowances for both these sources of fallacy will be made in the following analysis.

We may now consider this problem from another point of view. If the pairs arise entirely at random, then the interval between the dates of attack—or what we have to consider in our data, the dates of admission to hospital—will also form a chance distribution; that is to say, the number of pairs observed with any particular interval between

the dates of admission to hospital, will, within the limits of errors from random sampling, be proportional to the chances of pairs with such intervals occurring. Now it is clear that, if the period is limited to 180 days, a pair of 0's (i.e., both members of the pair admitted to hospital on the same day) may occur on 180 days, but a pair with a 179 days interval can only occur once, as the first member of the pair must be attacked on the first day of observation and the second on the last, so that the chance of a pair with an interval of 179 days would be 180 times as unlikely as a pair with no days interval.*

This statement would be strictly true only if the chance of contracting the disease was uniform throughout the period, and if no gaps were made in our series by deaths and repatriations. The former difficulty will be discussed more at length later, and the latter must be borne in mind when we come to interpret the deductions which can be drawn from these figures. This factor will also tend to reduce the total number of pairs recorded.

Table XXXI. gives the number of pairs tabulated according to days of interval between admission to hospital of each member of the pair, grouped in ten day periods. All the Natives from which these figures were obtained had been under observation for six months, and no pair occurring after this period is included, so that an interval of more than 182 days cannot arise. Groups of three or more Natives bearing consecutive numbers have been split into pairs; thus a group of three is considered as two pairs, a group of four as three pairs, and so on.

A study of this table shows that the "want of fit" of observation to theory is largely due to an excess of cases in the first group. We must, however, remember that the attack-rate is not uniform throughout the period of six months, and we shall obtain a much closer approximation to the true theoretical distribution if we make allowances of the variations in the attack-rate. The following method was suggested to me by Dr. Troup. If we divide the 124 pairs in the same ratio as the monthly attack-rate (see Table III.), we obtain the following groups:—31·9, 25·3, 19·8, 18·2, 16·5, and 12·3. Now let us calculate the distribution of $12 \cdot 3 \times 6$ pairs over the whole period,

* We can calculate the number of pairs to be expected in the following way:— Let n = number of days in the period, then $n + (n-1) + \ldots + 2 + 1 = m = \frac{n}{2}(n+1)$. a = number of pairs. Let it be required to find the number expected with intervals 0-9 days, 10-19 days, etc. Then,

$$T_{0-9} = \frac{a \times 10\,(2n-9)}{m} = \frac{10\,a\,(2n-9)}{n(n+1)}$$

and $$T_{10-19} = T_{0-9} \cdot \frac{2n-19}{2n-9} \quad \text{etc.}$$

TABLE XXXI.

Table giving days of interval between admission to hospital of each member of a pair as observed, also giving the most probable number and the value of χ^2 *for the difference.*

Days interval.	Observed number.	Probable number on law of chance.	χ^2
0- 9	25	13·3	10·2925
10-19	12	12·5	0·0200
20-29	16	11·8	1·4949
30-39	17	11·0	3·2727
40-49	10	10·2	0·0039
50-59	8	9·5	0·2368
60-69	5	8·7	1·5736
70-79	6	8·0	0·5000
80-89	4	7·2	1·4222
90-99	6	6·5	0·0385
100-109	2	5·7	2·4018
110-119	5	4·9	0·0020
120-129	2	4·2	1·1524
130-139	3	3·4	0·0471
140-149	2	2·8	0·2286
150-159	1	1·9	0·4263
160-169	0	1·1	1·1000
170-179	0	0·4	0·4000
Total	124		24·6133

P = 0·1043

and (16·5—12·3) or 4·2 × 5 pairs over the first five months, 1·7 × 4 pairs over the first four months, and so on. We shall then, by summing the number of pairs occurring with 0 to 9 days interval, etc., obtain a new distribution which will be the expected distribution when the varying monthly attack-rates have been allowed for. This has been done, and in Table XXXII. the contributions to the total number of expected pairs from each factor are shown in columns 1 to 6. The value of $\chi^2 = 11{\cdot}1725$, which leads to P = ·8, and shows that when the varying monthly attack-rates are allowed for, the distribution observed does not differ from theory by more than might reasonably be expected on the laws of random sampling.

TABLE XXXII.

Table giving the result of calculated distribution of pairs according to the monthly attack-rate.

Interval of days.	COLUMN.						Number of pairs expected by Theory.	Observation.	χ^2
	1	2	3	4	5	6			
0- 9	7·95	2·70	1·08	1·00	3·34	3·62	19·69	25	1·4259
10- 19	7·50	2·52	0·99	0·88	2·74	2·20	16·83	12	1·3714
20- 29	7·05	2·33	0·89	0·77	2·14	0·78	13·96	16	0·2857
30- 39	6·59	2·14	0·80	0·65	1·53		11·71	17	2·4009
40- 49	6·14	1·96	0·71	0·53	0·93		10·27	10	0·0087
50- 59	5·78	1·78	0·61	0·41	0·33		8·91	8	0·0910
60- 69	5·24	1·59	0·52	0·30			7·65	5	0·9467
70- 79	4·78	1·40	0·43	0·18			6·79	6	0·9412
80- 89	4·33	1·22	0·33	0·06			5·94	4	0·3529
90- 99	3·88	1·03	0·24				5·15	6	0·1588
100-109	3·42	0·84	0·14				4·40	2	1·1000
110-119	2·97	0·66	0·05				3·68	5	0·4568
120-129	2·51	0·47					2·98	2	0·3333
130-139	2·03	0·29					2·32	3	0·2130
140-149	1·61	0·10					1·71	2	0·0529
150-159	1·16						1·16	1	0·0333
160-169	0·70						0·70	0	0·7000
170-179	0·25						0·25	0	0·2500
	73·89	21·03	6·79	4·78	11·01	6·60	124·10	124	11·1225

P=0·8497

Unless, therefore, we are to consider that infection from case to case is as likely to occur with an interval of one day as with an interval of several months, there seems to be no justification for the assumption that the want of fit in the distributions given in Table XXVII. can be ascribed to infection. This suggestion of an indeterminate period for incubation seems hardly reasonable, and has, so far as I know, in this class of disease no parallel. The "want of fit" in these distributions is possibly due to varying attack-rates on different mine properties, combined with irregularities due to the fact that all the Natives in these groups had not been under observation for precisely the same period, and also that the attack-rate varies with the period of residence. The improvement in the "fit" of the distribution shown in Table XXXI. when these two latter factors are allowed for is, as we have seen, very marked.

Before leaving this question of the infectiousness of pneumonia

there is one other point to which attention may be drawn. We have already shown that the attack-rate of pneumonia is greatest on the arrival of Natives on the Rand, and gradually decreases as the period of residence increases (see Tables III. and IV.). The usual history of an outbreak of an infectious disease is of an entirely different character; the attack-rate as recorded day by day rises more or less rapidly for some days until the maximum is reached, when a steady, but somewhat slower, decline takes place. In the case of pneumonia amongst "Tropical Natives" we find the onset to be explosive in character, the attack-rate being at its maximum in the first twenty-four hours after arrival on the Rand, and thereafter falling rapidly: so that a curve showing the attack-rate according to length of residence on the "gold fields" is of the "J" type (see Charts I. and II.); such a curve would, I think, be unique as representing the history of an outbreak of an infectious disease.

In conclusion, a study of these figures shows definitely that if infection from case to case play any part in determining the prevalence of this disease among "Tropical Natives," it can be, at most, but a very small one; and moreover we can obtain no evidence of its existence. On the other hand, pneumococcal coryzas would appear, at least among Europeans, to be markedly infectious. The way in which catarrhal attacks often apparently associated with the pneumococcus, seem to spread from person to person throughout a household, or among the members of an office, strongly suggest case to case infection, nevertheless no such phenomena is apparent in the case of pneumonia. Nor, among these Natives, is pneumonia commonly preceded by catarrhal conditions of the naso-pharynx, or diseases of the influenzal type.

Finally, attention may be again drawn to the fact that this section deals only with the evidence in regard to infection from case to case among the Natives themselves. It has been suggested that the outbreak of pneumonia among these Natives is due to their infection with the pneumococcus contracted from Europeans with whom they associate when travelling to the mines. At present there is no direct evidence available in connection with this problem; it is one which will require investigation by other methods.

SECTION IV.

A consideration of The Effect of Prophylactic Inoculation of a Pneumococcal Vaccine on the Incidence and Mortality of Pneumonia.

A brief account only of the history of the preventive inoculation for pneumonia on the Rand need be given here. Full details of the bacteriological aspect of the investigation will no doubt be found in Sir Almroth Wright's expected report, and I shall confine myself to considering the results of some of the experiments from the statistical point of view.

Sir Almroth Wright, on the invitation of the Governing body of the Witwatersrand Native Labour Association, arrived in Johannesburg at the end of September, 1911. The first prophylactic inoculations were made on October 4th, in the W.N.L.A. compound; "Tropical Natives" only were treated. The method adopted was, first, to exclude from a gang any sick Natives, or those who appeared to be sickening for some illness, and then to divide the remainder into halves: the members of one half received a prophylactic inoculation, those of the other half being kept under observation as controls. These inoculations were made as soon as possible after the arrival of the Natives in the compound, and thus they were under the direct observation of Sir Almroth Wright and his assistants, for a period of about three weeks before being sent to work on the mines.

The first two gangs treated received a dose of vaccine of forty and sixty millions respectively; this vaccine was prepared in London. Subsequently a locally prepared vaccine was used, the doses being increased to 100, 150, and 200 millions. All inoculated Natives received two doses of vaccine unless they fell sick before the second was due. The interval between the injections varied from about seven to twelve days. In February the dose was increased to 300 millions. This series of inoculations I shall refer to as the "*first experiment.*"

While these inoculations were being carried out in the W.N.L.A. Compound large numbers of Natives were being similarly treated in some of the mine compounds along the Reef. This procedure has unfortunately made it impossible to satisfactorily follow the history of the experimental Natives leaving the W.N.L.A. Compound, for it was discovered that some of the controls were being inadvertently inoculated, and that inoculated Natives were being reinoculated, on

the mines. The inoculation of the controls after a period of residence on the Rand, and their transference from the group "control" to the group "inoculated" constituted a very serious source of error. This will be at once apparent when we recollect that the attack-rate falls rapidly with the increase in the period of residence; and consequently, even if the inoculation itself were of no value, such a transfer at any date subsequent to the commencement of the observations would give rise to an apparent correlation in favour of inoculation. As it appeared impossible to satisfactorily estimate the magnitude of this source of error, I have confined myself to dealing with the results of this investigation only in so far as they were observed in the W.N.L.A. Compound.

At the commencement of the following recruiting season for "Tropical Natives"—August, 1912—a further experiment was planned, the preparation of the vaccine, and the inoculation of the "boys," being conducted by Dr. Parry Morgan, one of Sir Almroth Wright's assistants. Approximately 8,000 Natives were dealt with, and I shall refer to this investigation as the "*8000 experiment.*" In this experiment again all "Tropical Natives" on arrival in the compound were divided into three groups—sick Natives were excluded, and given the distinguishing letter "F," and the remainder were divided into two classes by the inoculation of four and the omission of the fifth, who was regarded as a control and distinguished by the letter "E," the inoculated Natives being also given distinguishing letters according to the dose of vaccine they received. Every Native in this experiment was traced for a period of at least six months. After allotment, returns were received from the mines when any of these Natives were admitted to hospital, died, or were repatriated, together with the diagnosis made by the Medical Officer of the mine. I believe these returns are as exact as it is possible to get them, and they may be safely regarded as closely approximating to the real conditions. The doses of vaccine inoculated varied from 250 to 2,500 millions. A few much larger doses were also tried, mainly to determine if very large doses would give rise to toxic symptoms. The method of preparing the vaccine also varied, but it can be divided into two main classes: vaccine in which glucose was added to the medium in which the organism was grown, and that in which it was omitted. The main ingredients of the medium were broth with blood-serum and hæmoglobin, to which in some cases glucose was added. These two vaccines will be subsequently referred to as "Blood Vaccine" and "Glucose Vaccine."

The following table shows the nature of the vaccine, the dose, and the number of cases, in each group of this experiment.

TABLE XXXIII.

"8000 Experiment."
Results over period of six months.

Letter.	Nature of Vaccine	Dose in Millions.	Number in Group.	Mean pop. for six months.	PNEUMONIA. Cases.	Attack-rate per thousand.	Deaths	Case mortality per cent.
B	Blood	250	646	613	81	132	25	31
C	,,	500	759	722	90	124	26	29
A+C	,,	600°	162	158	17	108	2	12
D+H	Glucose	1250*	157	154	22	143	3	14
G	,,	500	463	440	46	104	19	41
H	,,	1000	650	627	69	110	17	25
J	,,	2500†	1661	1572	187	119	55	29
K	Blood	1500‡	1651	1564	175	112	55	31
Q	Various	Odd 200 —40000	75	72	7	97	3	43
E	Controls	0	1545	1439	231	160	74	32
F	Omitted from experiment	0	354					

° First dose 100, second 500.
* First dose 250, second 1,000.
† Doses varied from 2,400 to 3,000, majority 2,500.
‡ Doses varied from 1,250 to 1,875, majority 1,500.

A general inspection of this table shows, first, that the attack-rate was highest amongst the controls, but that the case mortality in this group is not higher than that of several of the inoculated groups. Owing, however, to the small numbers comprised in many of these groups errors due to sampling will be considerable, and to estimate properly the effect of vaccination we must adopt other methods than those of mere inspection of the figures and rates. The effect of inoculation can be studied in relation to both the attack-rate and the death-rate from pneumonia. Any modification of the severity of the attack can be measured either by the case mortality, or by the length of the illness, if it were possible to obtain uniform data in regard to this latter point. The period of stay in the mine hospital was stated in the returns received from the mines, but on enquiry it became evident that this information would not be of any value for the purpose of determining the severity of the attack, because in many mine hospitals no case of pneumonia is discharged under a

minimum period. Any results reached by considering the period spent in hospital (as regards the returns from the mines) will, for this reason, be unsatisfactory.

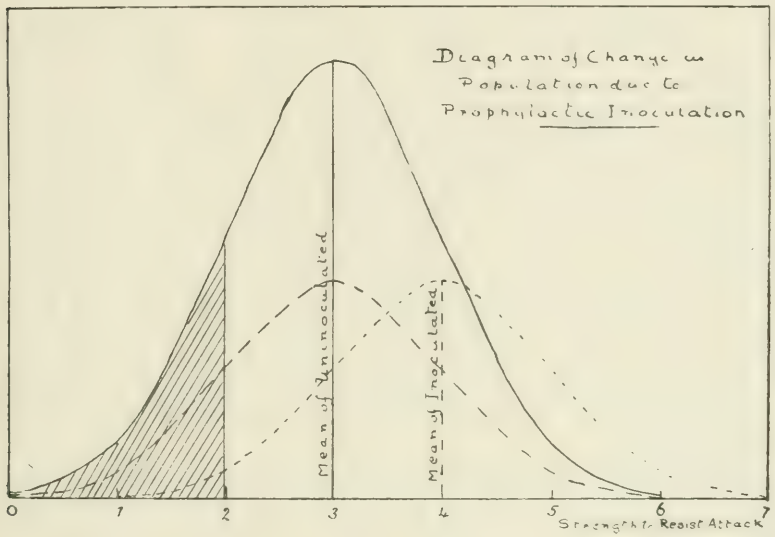
We have, therefore, three measures by which we can estimate the effect of prophylactic inoculation against pneumonia; namely, (1) the attack-rate, (2) the death-rate, and (3) the case mortality from this disease. The question arises as to what statistical methods we shall employ in order to obtain the best guide to the results of this procedure. One naturally turns first of all to the "four-fold table"[25] method of Professor Pearson, the tetrachoric r, (r_t) as it is now called. This method has been much used for similar material in relation to vaccination against smallpox, anti-typhoid inoculation, and other enquiries of the same nature. Recently, however, Mr. G. U. Yule has attacked the use of this method for dealing with such problems,[15] and has suggested as preferable the employment of certain "coefficients of association." A full reply to these criticisms of Mr. Yule, and a demonstration that his suggested measures are unsuitable for problems such as those before us, has been made by Professor Pearson and Dr. Heron.[16] The objection to the tetrachoric r is that this method assumes that the distribution of the variates is continuous, and that they are Gaussian in type. It has, however, been shown that a very considerable departure from normality does not seriously affect the results obtained by this method, provided that the dichotomies are not taken too near the tails of the distribution. The nature of our distributions is unfortunately such, that the divisions in many instances occur in such a situation that only small frequencies are obtained in two of the cells. These inevitably small frequencies constitute an objection to the use of this method for solving these problems, because we are unable to *assert* that the distribution of our variates is approximately Gaussian in type. Mr. Yule would, apparently, even consider them discontinuous; for as he says, A man is either "dead" or "not dead"; and presumably also either "attacked" or "not attacked" by any specific disease. This is a question of fundamental importance; and although it has been fully answered by Pearson and Heron in the paper just referred to, and was also dealt with by Greenwood at the time of its enunciation,[17] yet it would appear desirable to deal briefly with it here: for it is apparently one which presents difficulties to medical readers, to whom the literature referred to may not always be readily accessible.

Let us consider the problem in regard to recoveries and deaths in a population of those attacked by a disease. We wish to measure the virulence of an outbreak of the disease by the ratio of recoveries to deaths. Are we then to consider the distribution continuous or discrete? If we group our data into "dead" and "not dead," and

consider the groups as discrete, and differing only by the presence or absence of a character (life), we are assuming that the disease can be best represented on the supposition that it has only two grades of virulence. If on the other hand we regard virulence as a continuous variate we shall allow for every grade of severity in attack, and consider the dividing line (death) merely to cut this distribution at some particular place. The fact that the continuous distribution is assumed to continue after the position of the dichotomy appears to constitute a difficulty to some. The medical reader will perhaps more easily appreciate the conception of continuity by means of a simple illustration. Let us imagine a number of pellets, shot from a fixed air gun, to travel exactly in the same direction, they will not, nevertheless, all come to rest at the same spot; but their positions can when enough have been discharged be represented by some continuous curve. If they were allowed to fall upon a tray divided into compartments we should probably find that in the first compartment a stray pellet or so was present, the number in subsequent compartments increasing regularly up to a certain compartment and thereafter decreasing. That is to say, we must consider the velocity of the pellets as a continuous function, influenced it may be by many factors. If we introduce a sheet of paper at any position on the range so that some of the pellets fall short of the paper whilst others pierce it, it would not now be justifiable—in fact it would be quite misleading—to consider that our distribution could be better considered as discrete and divisible into two classes—those that "pierce" and those that do "not pierce" the paper. Clinical observation teaches us that we have in most diseases a range of all degrees of severity, from mere indisposition up to death, and to assume that a break in the continuity of the range occurs at death, or that the range terminates abruptly at this point is, I think, unscientific, particularly as experience shows that while some people appear only just to fail to overcome the disease, others have seemingly from the commencement no hope of recovery. It has been shown that in some diseases at any rate, the normal curve may as a first approximation be used satisfactorily to describe such distributions. Even if a Type I. curve, such as those shown in Charts VII. and VIII., is a more usual form than the Gaussian, very little error will be introduced in the value of r_t by assuming the distribution to be normal, unless the dichotomies are made at the extreme limits of the range.

It seems to me that the "four-fold" table method would therefore be quite satisfactory for ordinary use in regard to the variate "recovery and death," or "attacked and not attacked." But in regard to the other variate, more difficulty arises: are we to consider "inoculated and uninoculated" as represented by a continuous

distribution of more or less Gaussian type? We desire to measure the protection conferred by inoculation. My conception of the problem is as follows: Members of a population possess the character "power to resist attack" in varying amounts, and the distribution of this population in regard to the character is continuous and probably capable of being represented, at least approximately, by a normal curve. Assuming inoculation to increase this "power to resist attack," its effect will be to bodily move the treated portion of this population in the direction of increasing amounts of this power, without, necessarily, altering the type of the distribution.



The accompanying diagram illustrates this suggestion. The continuous curve represents the original population in respect to the factor "power to resist attack." The broken curves are supposed to represent the population after one half has received a prophylactic dose of some vaccine. The curve representing the inoculated population has been moved to the right, but is otherwise unaltered. The shaded portion of the diagram, ending at the ordinate "2," is supposed to represent diagramatically the portion of the population who are attacked. It will be seen that the effect of shifting the curve representing the inoculated population to the right, is to reduce the area which falls in the shaded portion of the diagram, i.e., to reduce the number of the population who contract the disease.

If this conception of the effect of inoculation be correct, the amount of shift in the position of the mean would give us a measure

of the protection conferred by inoculation. If the distributions were truly Gaussian the displacement in the mean of the inoculated population could easily be determined from a table of the "Probability Integral" when the attack-rates were known. But unfortunately we cannot *assert* that the distributions are Gaussian, and large errors may arise when the attack or death rates are small, if the distribution should not be so. Further, when only small areas at the tail are relied on for calculating the position of the mean errors of random sampling, unless very large total numbers are being dealt with, may introduce serious errors.

Again, the two sub-distributions might be considered as concentrated at their means, and then regarded as differing by a unit "something" from each other, with a mean equidistant from each group when so concentrated. If we equalise the groups, we could by using the "New Correlation Method" described by Professor Pearson,[23] rapidly calculate the correlation. This method of equalising the sub-groups is arbitrary, and has not, I think, any theoretical justification; nevertheless, it has the advantage of enabling an approximate value of the correlation to be found in a few moments if a copy of Sheppard's "Tables of the Probability Integral"[26] be at hand. In a few of the following tables the value of "r" thus obtained has been added (under the symbol r_k); it will be noticed that this value rarely differs by more than the probable error from the value of r_t. So close an agreement in the two values does not always occur, and in a few experimental examples where the value of r_t was large the values differed by considerably more than the probable error.

Considering these difficulties, which I have discussed at some length because of their intrinsic importance in medical problems, we cannot consider the "four-fold" method as entirely satisfactory for our present purpose, nevertheless, if a correlation value is desired it is, I believe, the best method at present available for this purpose. Fortunately, however, we can also obtain a direct measure of the improbability that our observed distributions have arisen by errors of random sampling, without making any supposition as to the nature of the distribution, or even as to whether the variates are continuous or discrete. To determine this value the "goodness of fit"[14] is calculated, the value of P for given χ^2 for $n'=4$ is rapidly obtained from Elderton's Tables.[22] When, however, P is very small, it is impossible to grasp its significance, a difficulty which has been met by Professor Pearson by the introduction of a *correlation scale of the improbability of independence*.[18] This constant is denoted by the symbol γ.

In the analysis of the following tables I have calculated the tetrachoric "r" (r_t) because, although not perfect for this purpose

it is useful as a control, and is also useful for purpose of comparison with similar problems in which it has previously been employed. Everett's Tables of Tetrachoric Functions[24] were used, and the probable error calculated by the method given by Pearson.[21] The probability of the variations obtained having arisen by chance was also calculated by the method of "goodness of fit," and is given in each case, and in addition, in some instances, the value of "r_p" as mentioned above, $_{\sigma}r_r$ being determined by the aid of Professor Pearson's tables, and r_r then obtained directly from Mr. Soper's Abac.[18] In the calculation of χ^2 the theoretical values were taken to the nearest unit.

Before turning to the actual results obtained by these methods, it will be well to consider briefly the meaning of the word "significant" when applied to a correlation coefficient; that is to say, the interpretation of a result in relation to its probable error. It is no doubt a matter of opinion, to a certain extent, as to what degree of probability we should demand before a value can rightly be considered as significant. No reasonable person would consider, that a variation in a distribution which is as likely as not to arise owing to errors of random sampling was significant evidence of the effective introduction of any new factor in the causation of the variation. If, however, the odds against a certain distribution arising by chance are, let us say, four to one, can we then consider such a result as significant of the introduction of a new factor? Clearly not! although such a result might be considered as indicating a certain "a priori" reason for repeating the experiment, yet no deductions as to its probable influence could be legitimately drawn from such a result. The odds which we should demand before considering a result as significant must be a matter for individual judgment, and those who have had most experience of this kind of analysis will be best able to form a reliable estimate of the significance, or otherwise, of any particular value. The usual practice amongst biometric workers is to consider a coefficient of correlation as probably significant if its value is greater than three times the value of its probable error. For general purposes this is probably a fairly safe measure, but it should be remembered that even a guide of this sort, although showing that the chances are certainly in favour of the observed correlation being significant, does not constitute a definite proof that it is so, and all collateral evidence must receive due weight. It is, however, in this sense that the term "significant" is used in this paper.

First, let us deal with the results of what we have termed the "first experiment." The data in regard to the number attacked with pneumonia are only available for the first few weeks after inoculation. The number attacked in each group, inoculated and uninoculated, is shown in this table.

TABLE XXXIV.

	Inoculation. +	—	Total.	
Not attacked	5822	5494	11316	$r_t = \cdot0876 \pm \cdot0196$
Attacked	149	195	344	$\chi^2 = 8\cdot73917 \quad P = \cdot0337$
				$r_p = \cdot06 \quad r_k = \cdot07$
Total	5971	5689	11660	

There is a small but appreciable correlation in favour of inoculation, as reducing the attack-rate; and the chances are 29 to 1 against so large a variation from the expected arising owing to errors of random sampling.

If we employ the number of deaths, instead of the number attacked, as the measure of the effect of the inoculation, we obtain the following results:—

TABLE XXXV.

	Inoculation. +	—	Total.	
Not dead	5922	5602	11524	$r_t = \cdot1475 \pm \cdot0268$
Dead	49	87	136	$\chi^2 = 13\cdot13498 \quad P = \cdot0044$
				$r_p = \cdot13 \quad r_k = \cdot12$
Total	5971	5689	11660	

Here the odds against such a variation from the expected as this arising by chance are 227 to 1. We may, therefore, regard both these correlations, although small, as significant.

A table of recoveries and deaths amongst the inoculated and uninoculated in this group can be constructed, and is as follows:—

TABLE XXXVI.

	Inoculation. +	—	Total.	
Recoveries	100	108	208	$r_t = \cdot1909 \pm \cdot0567$
Deaths	49	87	136	$\chi^2 = 4\cdot95219 \quad P = \cdot1761$
Total	149	195	344	

In this table the value of r_t is, according to our definition, just significant, and the odds against a variation as large as this arising by chance are only 4·7 to 1.

Finally, from the figures obtained from this experiment we can form a "three by two fold" table, as under:—

TABLE XXXVII.

	Inoculation. +	—	Total.	
Not attacked ...	5822	5494	11316	
Attacked, recovered...	100	108	208	$\chi^2 = 12{\cdot}96818$ $P = {\cdot}0237$
Attacked, died ...	49	87	136	
Total	5971	5689	11660	

The chances are, therefore, about 42 to 1 against this distribution having arisen owing to errors of random sampling.

Turning now to an examination of the data obtained from the "8000 experiment," the returns from which are available for a period of six months, we can construct the following tables. In these tables the *average* number of Natives exposed to risk for the period dealt with is used, not the number *actually* inoculated, because some of the Natives died from other causes and some were repatriated during the period of observation. Tables showing the number attacked and the number who died are given below.

TABLE XXXVIII.

	Inoculation. +	—	Total.	
Not attacked ...	5228	1208	6436	$r_t = {\cdot}1132 \pm {\cdot}0180$
Attacked	694	231	925	$\chi^2 = 19{\cdot}64246$ $P = {\cdot}0002$
				$r_p = {\cdot}10$ $r_k = {\cdot}10$
Total	5922	1439	7361	

TABLE XXXIX.

	Inoculation. +	—	Total.	
Not dead	5717	1365	7082	$r_t = {\cdot}1070 \pm {\cdot}0260$
Dead	205	74	279	$\chi^2 = 8{\cdot}49944$ $P = {\cdot}0377$
				$r_p = {\cdot}06$ $r_k = {\cdot}09$
Total	5922	1439	7361	

These results confirm those already found from the "first experiment," in that they show that whether we employ the attack-rate or the death-rate as the measure of the protection afforded by inoculation, a small, but definite correlation in favour of this process is observed in all four tables.

TABLE XL.

	Inoculation. +	—	Total.	
Recoveries	489	157	646	r_{ρ} = ·0124 ± ·0397
Deaths	205	74	279	χ^2 = 0·43750 P = ·9130
Total	694	231	925	

In regard to the effect of inoculation on the case mortality the results here are clearly not significant. In a similar table constructed from the data of the "first experiment" we found a higher correlation, but as the value of P was ·1761, it is not therefore very surprising to find a lower value here; nevertheless, as the correlation was just "significant" in the former table a further examination was undertaken. It seemed possible that the explanation of the difference in values might lie in the fact that the returns for the "8000 experiment" dealt with a six-month period, whereas the former were only concerned with a mean period of approximately four weeks from the date of inoculation, and if the beneficial influence in this respect were only of temporary duration its effect might be masked in the latter table. To estimate the value of this suggestion the following table was constructed by including only the cases which arose in the W.N.L.A. compound during the first month after inoculation.

TABLE XLI.

Case Mortality.

	Inoculation. +	—	Total.	
Recoveries	105	32	137	r_{ρ} = ·1085 ± ·0742
Deaths	71	29	100	χ^2 = 0·81315 P = ·8384
Total	176	61	237	

Here again, although the absolute value of the correlation is increased it cannot in relation to its probable error be considered as significant, and the value of P indicates that variations as great as this are to be expected in samples of this size. It seems, therefore, that while we can state that prophylactic inoculation has a value in diminishing the attack-rate from pneumonia, there is at present no definite evidence that it has much influence in lowering the case mortality. It is possible that larger numbers when available may definitely settle this point, but so far as the present returns go we have only a suggestion

that a small reduction in the case mortality follows preventive inoculation, and this possibly is most marked for a short period following inoculation. In this connection one point remains to be noted; the large majority of Natives comprised in the inoculated group of the "first experiment" received two comparatively small doses of prophylactic vaccine, while those composing this group in the "8000 experiment," with a few exceptions, received only one dose. Whether this factor had any effect on the result further observation can alone definitely determine, the following considerations may, however, be of some interest.

A reference to Table XXXIII. shows that the two groups, in the "8000 experiment," which received two doses of prophylactic vaccine, viz.: (A+C) and (D+H) had a lower case mortality than any of the other groups; but the number of cases concerned is so small that no reliance can be attached to such results, and more especially so when we remember how very variable the case mortality from this disease is liable to be. It was, nevertheless, suggestive; and I have, therefore, tabled the records of 146 cases of pneumonia treated in the W.N.L.A. Hospital at the time of the "first experiment," and have grouped them according to whether they had received two or only one dose of prophylactic vaccine.

TABLE XLII.

	Inoculation.			
	+	++	Total.	
Recovered	55	44	99	$r_i = \cdot2631 \pm \cdot0867$
Died	18	29	47	$\chi^2 = 3\cdot13883 \quad P = \cdot3736$
Total	73	73	146	

This correlation, if considered as significant, is in favour of the cases which received only one prophylactic dose of vaccine. There are two possibly disturbing factors to be considered, (1) twenty-seven of the cases in the first group, as against twenty-two in the second, were *treated* with a pneumococcal vaccine; this, as we shall see later, would, if it had any effect, tend to reduce the correlation, and consequently the observed value would be too small: (2) the cases appearing in the first group occurred, on the average, sooner after arrival in the compound than those in the second group. This was due to the fact that, with very few exceptions, all these Natives received two inoculations, unless they had fallen sick in the interval after the first dose and before the second was due. So that cases among the inoculated occurring within a week or ten days of arrival will appear in the first group and subsequent cases in the second group. We must recollect, however, that the case mortality does not appear to be

a function of the period of residence on the Reef; and if this factor has any influence, it may be due to the fact that the cases in the first group occur on the whole with a shorter interval between inoculation and attack than those in the group with two prophylactic inoculations. Although, as previously stated, the majority of Natives comprising the "first experiment" received two doses of prophylactic vaccine, and those in the "8000 experiment" only one, yet no satisfactory conclusions in respect to the relative value of one or two doses can be drawn from the case mortality rates in the two groups. First, because many of the attacked in the earlier part of the period —the only portion for which we have reliable records—had, as just explained, only received the first dose; secondly, the season during which they were under observation was not the same, and the case mortality varies considerably from time to time; thirdly, the doses of the vaccine given are not comparable in the two experiments.

The "three by two fold" table for the "8000 experiment." corresponding to that given for the "first experiment," is as follows:—

TABLE XLIII.

	Inoculation.			
	+	—	Total.	
Not attacked ...	5228	1208	6436	
Recovered	489	157	646	$\chi^2 = 20{\cdot}120392$ $P = {\cdot}0011$
Died	205	74	279	
Total	5922	1439	7361	

Thus only once in a thousand samples should we expect a result as bad as this if the variations were due to errors of sampling.

Having seen that a certain amount of protection against attack is conferred by inoculation, we must proceed to investigate the period for which such protection lasts. Table XLIV. gives the monthly details in regard to the number attacked, and the number who died (with the rates) among the inoculated and controls comprised in the "8000 experiment."

This table was constructed by placing in the group 1st month all cases of pneumonia which occurred within thirty days of the date of inoculation, and so on; and, the deaths in each group refer to deaths among the attacked in each month after inoculation. Thus although a Native may have died on the fortieth day from the date of inoculation he will appear as a death in the first month if the disease was contracted within thirty days from inoculation.

TABLE XLIV. A.

Column 0 = Month after arrival, 1 = Average population, 2 = Pneumonia cases, 3 = Pneumonia deaths, 4 = Other respiratory diseases cases, 5 = Other respiratory diseases deaths, 6 = Other medical diseases cases, 7 = Other medical diseases deaths, 8 = Repatriations.

	INOCULATED.								UNINOCULATED (CONTROLS).							
0	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8
1st	6170	179	71	109	2	273	38	2	1523	68	29	42	2	70	12	1
2nd	6079	140	34	194	3	369	13	18	1488	52	15	48	1	94	1	11
3rd	6000	116	23	185	2	362	6	60	1459	33	8	45	0	105	2	19
4th	5882	100	33	138	1	276	11	98	1425	34	9	39	1	65	1	29
5th	5757	90	22	102	5	235	8	72	1386	28	5	37	0	56	1	31
6th	5648	69	22	114	2	187	10	76	1356	16	8	37	1	45	2	15
Total	5922	694	205	842	15	1702	86	326	1456	231	74	248	5	435	19	106
7th	2556	36	12		3		2	34	649	9	5		1		1	11
8th	2518	31	6		0		0	20	637	7	0		0		1	6
9th	2492	20	7		1		4	13	628	8	4		1		0	6

TABLE XLIV. B.

Rates.

Column 0 = Month after arrival, 9 = Pneumonia attack-rate per thousand, 10 = Pneumonia death-rate per thousand, 11 = Pneumonia case-mortality per cent., 12 = Respiratory diseases death-rate per thousand, 13 = Respiratory diseases case-mortality per cent., 14 = Other medical diseases death-rate per thousand, 16 = Other medical diseases case-mortality per cent., 16 = Repatriations per thousand.

	INOCULATED.								UNINOCULATED (CONTROLS).							
0	9	10	11	12	13	14	15	16	9	10	11	12	13	14	15	16
1st	29·0	11·5	39·7	0·32	1·8	6·16	13·9	0·32	44·6	19·0	42·6	1·31	4·8	7·88	17·1	0·66
2nd	23·0	5·6	24·3	0·49	1·5	2·14	3·5	3·0	34·9	10·1	28·8	0·67	2·1	0·67	1·1	7·4
3rd	19·3	3·8	19·8	0·33	1·1	1·00	1·7	10·0	22·6	5·5	24·2			1·37	1·9	13·0
4th	17·0	5·6	33·0	0·17	0·7	1·87	4·0	16·7	23·9	6·3	26·5	0·70	2·6	0·70	1·5	20·4
5th	15·6	3·8	24·4	0·87	4·9	1·39	3·4	12·5	20·2	3·6	17·9			0·72	1·8	22·4
6th	12·2	3·9	31·9	0·35	1·8	1·77	5·4	13·5	11·8	5·9	50·0	0·74	2·7	1·47	4·4	11·1
Av. monthly rate	19·5	5·8		0·42		2·4		9·2	26·4	8·4		0·57		2·20		12·2
7th	14·1	4·7	33·3	1·17		0·78		13·3	13·9	7·7	55·6	1·54		1·54		16·9
8th	12·3	2·4	19·4					7·9	11·0					1·57		9·4
9th	8·0	2·8	35·0	0·40		1·61		5·2	12·7	6·4	50·0	1·59				9·6

An inspection of this table shows that for the first two months the attack-rate amongst the inoculated is considerably lower than amongst the controls. Four-fold tables for the attack-rate for each month have been prepared, and are as follow:—

TABLE XLV.

A—1st month.

	Inoculation. +	—	Total.	
Not attacked ...	5991	1455	7446	r_l = ·1138 ± ·0268
Attacked	179	68	247	χ^2 = 9·49594 P = ·0221
Total	6170	1523	7693	

B—2nd month.

	Inoculation. +	—	Total.	
Not attacked ...	5939	1436	7375	r_l = ·1053 ± ·0296
Attacked	140	52	192	χ^2 = 6·56665 P = ·0891
Total	6079	1488	7567	

C—3rd month.

	Inoculation. +	—	Total.	
Not attacked ...	5884	1426	7310	r_l = ·0373 ± ·0325
Attacked	116	33	149	χ^2 = 0·69897 P = ·8611
Total	6000	1459	7459	

D—4th month.

	Inoculation. +	—	Total.	
Not attacked ...	5782	1391	7173	r_l = ·0808 ± ·0332
Attacked	100	34	134	χ^2 = 3·11217 P = ·3770
Total	5882	1425	7307	

E—5th month.

	Inoculation. +	—	Total.	
Not attacked ...	5667	1358	7025	r_l = ·0596 ± ·0357
Attacked	90	28	118	χ^2 = 1·37287 P = ·7159
Total	5757	1386	7143	

F—6th month.

	Inoculation. +	—	Total.	
Not attacked ...	5579	1340	6919	$r_f = -\cdot0077 \pm \cdot0406$
Attacked	69	16	85	$\chi^2 = 0\cdot00000$ $P = 1\cdot00000$
Total	5648	1356	7004	

These tables show that after the first two months there is very little evidence in favour of the protective influence of this vaccination, but as the number of the controls selected was small, variations in rates due to small samples is considerable. The variation in rates is shown graphically in Chart XIA., where it will be noticed how much more smoothly the rates run for the inoculated than for the uninoculated; the former are based on approximately four times the number of cases. Returning to the actual figures we notice that for the 6th month, and so far as the figures are available for the 7th and 8th months, the attack-rates among the uninoculated are actually a little less than among the inoculated. These differences are clearly not significant, and are again reversed in the 9th month. We can, I think, sum up this part of the evidence by saying that these returns indicate that any protection obtained lasts for only a limited period after inoculation, and that the general trend of the figures suggests that it does not entirely disappear until about the fifth month, although there is but little positive evidence that much of its efficacy remains after the second month. This falling off is even more marked when we exclude second attacks from the returns, as is shown in Chart XIB.

The prevalence of second attacks of pneumonia occurring among the inoculated and the controls is of some interest. We have already seen—on the basis of our previous definition of "second attacks"—that one attack does not apparently confer any immunity against a second. From the figures given below we see that a higher value is obtained for the correlation for immunity against second attacks amongst the inocluated, than that for the general attack-rate.

TABLE XLVI.

	Inoculation. +	—	Total.	
First attack only ...	514	146	660	$r_f = \cdot2655 \pm \cdot0532$
Second attack ...	50	33	83	$\chi^2 = 12\cdot5328$ $P = \cdot0061$
				$r_p = \cdot23$ $r_k = \cdot22$
Total recoveries from first attack ...	564	179	743	

TABLE XLVII.

	Inoculation. +	—	Total.	
Recovery 2nd attack...	37	26	63	$r_i = -\cdot0955 \pm \cdot1283$
Death in	13	7	20	$\chi^2 = 0\cdot27169$ $P = \cdot9460$
Total	50	33	83	

The table for the attacks is constructed from all recoveries from first attacks, and is not limited to a six-month period. As the period of exposure to risk of a second attack varied the calculation of rates, except for the comparison of the one group with the other, will be without value. Although the second attack-rate amongst the inoculated appears to be considerably reduced there is no evidence that the case mortality is favourably affected. An examination of the length of the interval between the first and second attacks would seem to suggest that the diminution in the rate among the inoculated is chiefly confined to a period of thirty days after the primary attack. The figures when thus sub-divided are very small, and consequently but little weight can be attached to this observation unless confirmed by future records.

The question of the effect of the variations in the size of the dose of protective vaccine can now be considered. The inoculated Natives are divided into two groups, (1) those receiving medium doses, viz., the sub-groups B, C, (A+C), G, H, and (H+C), and (2) those classed as large doses, viz., sub-groups J, K, and Q. These two groups, even when the question of dose is excepted, are not strictly comparable, owing to the fact that their arrival on the Rand did not take place at the same time of the year, and the attack-rate shows, as we have seen, a seasonal variation. As a check on these results it is advisable, therefore, to divide the controls into two groups, to correspond with those for the inoculated.

TABLE XLVIII.

	Inoculated (size of dose). Large.	Medium.	Total.	
Not attacked ...	2841	2387	5228	$r_i = \cdot0153 \pm \cdot0180$
Attacked	369	325	694	$\chi^2 = 0\cdot42096$ $P = \cdot9163$
Total	3210	2712	5922	

TABLE XLIX.

	Controls to Large.	Medium.	Total.	
Not attacked ...	629	579	1208	r_t = ·0857 ± ·0334
Attacked	106	125	231	χ^2 = 2·97172 P = ·3967
Total	735	704	1439	

Neither of these results can be considered as really significant, but any evidence in favour of the group "large dose" is more marked among the controls than the inoculated.

If the material be grouped according to the size of the dose—taking 250 millions as the unit for the group—the following table can be constructed from which a correlation value can be found by the new method described by Professor Pearson.[23]

TABLE L.

Dose of vaccine ...	250	500	750	1000	1250	1500	1750	2000	2500	Total.
Not attacked ...	532	1167		558	132	1391			1386	5166
Attacked	81	153		69	22	175			187	687
Total	613	1320		627	154	1566			1573	5853

r = —·0164 + ·0157* χ^2 = 3·27686 P = ·9850

In this table the Natives comprising the group "Q" are omitted. We again obtain what is practically a zero correlation between the size of dose and freedom from attack. The rates are naturally above the mean in the groups 250, 1,250, and 2,500, and below it in groups 500, 1,000, and 1,500; while the variations are not greater than, or even so great as, might be expected to arise from errors of random sampling. The case mortality is also, so far as these figures go, uninfluenced by the size of the dose, thus:—

TABLE LI.

	Inoculated (size of dose). Large.	Medium.	Total.	
Recoveries	256	233	489	r_t = —·0423 ± ·0424
Deaths	113	92	205	χ^2 = 0·45766 P = ·9090
Total	369	325	694	

* The probable error has been assumed to be $2\left(.67449\frac{(1-r^2)}{\sqrt{N}}\right)$

TABLE LII.

	Controls to Large.	Medium.	Total.	
Recoveries	72	85	157	$r_t = -\cdot0013 \pm \cdot0727$
Deaths	34	40	74	$\chi^2 = 0\cdot00000$ $P = 1\cdot0000$
Total	106	125	231	

It has already been pointed out that the method of preparing the vaccine was varied by the addition, in some cases, of glucose to the medium in which the organism was grown. The returns can be analysed in respect of this point as follows:—

TABLE LIII. *(attack-rate).*

	Variety of vaccine. Blood.	Glucose.	Total.	
Not attacked ...	2696	2469	5165	$r_t = -\cdot0096 \pm \cdot0181$
Attacked	363	324	687	$\chi^2 = 0\cdot10577$ $P = \cdot9790$
Total	3059	2793	5852	

TABLE LIV. *(case mortality).*

	Variety of vaccine. Blood.	Glucose.	Total.	
Recoveries	255	230	485	$r_t = -\cdot0133 \pm \cdot0427$
Deaths	108	94	202	$\chi^2 = 0\cdot02815$ $P = \cdot9944$
Total	363	324	687	

The "Q" group is omitted from these tables as mixed vaccines were given to its members. In both these tables the correlation is virtually zero, and there is, therefore, no evidence that the addition of glucose to the culture media had any effect on the results.

The morbidity and mortality returns obtained in regard to the Natives constituting the "8000 experiment" included other diseases as well as pneumonia, we can, therefore, proceed to consider the influence of the prophylactic inoculation in relation to "other medical diseases."

Taking first the whole group "all medical diseases other than pneumonia," the morbidity table for the six months is as follows:—

TABLE LV.

	Inoculation. +	—	Total.	
Not attacked ...	3378	761	4139	r_t = ·0598 ± ·0142
Attacked	2544	678	3222	χ^2 = 8·08589 P = ·0446
Total	5922	1439	7361	

In all the morbidity tables throughout this memoir it must be remembered that the number of attacked does not represent the number of *individual patients* admitted to hospital but the number of cases admitted; the same "boy" may have been, and, as a matter of fact was, particularly in regard to "other medical diseases," frequently readmitted on one or more occasions. It will be noted that a small but significant correlation in favour of inoculation is obtained from this group in relation to the attack-rate, but a zero correlation is obtained from the following mortality table.

TABLE LVI.

	Inoculation. +	—	Total.	
Not dead	5821	1415	7236	r_t = —·0052 ± ·0348
Dead	101	24	125	χ^2 = 0·00000 P = 1·0000
Total	5922	1439	7361	

These returns can be further sub-divided into respiratory and non-respiratory diseases. Under the heading respiratory diseases, cases reported as colds, bronchial catarrh, broncho-pneumonia, coughs, influenza, tuberculosis, etc., are classified.†

The morbidity and mortality tables for Respiratory diseases, excluding Lobar Pneumonia, are as under:—

TABLE LVII.

	Inoculation. +	—	Total.	
Not attacked ...	5080	1196	6276	r_t = ·0657 ± ·0173
Attacked	842	243	1085	χ^2 = 6·60737 P = ·0875
Total	5922	1439	7361	

† Pulmonary tuberculosis accounted for in all sixty-two cases, fifteen of which died. Three-quarters of the deaths from Respiratory diseases other than pneumonia were, therefore, attributable to tuberculosis.

TABLE LVIII.

	Inoculation. +	—	Total.	
Not dead	5907	1434	7341	r_t = ·0645 ± ·0706
Dead	15	5	20	χ^2 = 0·32116 P = ·9362
Total	5922	1439	7361	

Here again we obtain a correlation in favour of the inoculated in regard to the attack-rate from these diesases, but an insignificant value in regard to the death-rate. It will be observed that the case mortality in this group of diseases is low, less than 2 per cent. The number of deaths dealt with is so small that no conclusions of value can be drawn from this table. On sub-dividing the morbidity returns into (1) cases which occurred in the W.N.L.A. compound, i.e., early cases, and (2) cases reported from the mines, a drop in the value of the correlation corresponding to the lapse of time after inoculation is observed, a phenomenon similar to that found in the tables constructed from the pneumonia rates. The following are tables showing attack-rate from Respiratory diseases excluding pneumonia:—

TABLE LIX.

(Cases occurring in the W.N.L.A. compound.)

	Inoculation. +	—	Total.	
Not attacked ...	5876	1418	7294	r_t = ·1503 ± ·0432
Attacked	46	21	67	χ^2 = 6·17045 P = ·1048
Total	5922	1439	7361	

TABLE LX.

(Cases occurring on the mines.)

	Inoculation. +	—	Total.	
Not attacked ..	5158	1230	6388	r_t = ·0425 ± ·0179
Attacked	764	209	973	χ^2 = 2·72033 P = ·4422
Total	5922	1439	7361	

Very little weight can be attached to these values, nevertheless, so far as they go, they indicate a fall in the value of the correlation with the increase of time after inoculation.

The group "other medical diseases, excluding pneumonia," and "other respiratory diseases," yield zero correlations, and the probability is that the observed divergencies from the most probable distributions are merely due to errors of random sampling.

The following tables show the morbidity and mortality returns from other medical diseases excluding pneumonia and respiratory diseases.

TABLE LXI.

	Inoculation. +	—	Total.	
Not attacked ...	4220	1004	5224	$r_t = \cdot0248 \pm \cdot0150$
Attacked	1702	435	2137	$\chi^2 = 1{\cdot}21132$ $P = \cdot7529$
Total	5922	1439	7361	

TABLE LXII.

	Inoculation. +	—	Total.	
Not dead	5836	1420	7256	$r_t = -\cdot0233 \pm \cdot0369$
Dead	86	19	105	$\chi^2 = 0{\cdot}06264$ $P = \cdot9860$
Total	5922	1439	7361	

It would appear then that while prophylactic inoculation has apparently some influence in lowering the attack-rate from respiratory diseases other than lobar pneumonia there is no evidence that it has any influence in respect to non-respiratory diseases. This influence on respiratory diseases may be a measure of the prevalence of pneumococcal infections in this group. We know that in catarrhal conditions of the naso-pharynx the pneumococcus can frequently be recovered from the secretions in almost pure culture. In some cases of broncho-pneumonia it can be obtained by lung-puncture, and may also be found in many cases in the sputum. It is of interest in this connection to record that Dr. Watkins-Pitchford informs me that during the last year the pneumococcus has been obtained in practically pure culture from nearly all cases of so-called "Influenza" examined in the routine laboratories of the Institute, and that the influenza bacillus has been but rarely seen; in previous years, however, the influenza bacillus was frequently recovered from such cases.

Summarising the results obtained by this analysis of the experimental prophylactic inoculation with pneumococcal vaccine, we find:

(1) That the attack-rate from pneumonia is apparently lessened by inoculation, a small positive correlation being obtained.

(2) That the beneficial results observed are most marked during the first month after inoculation, and that they gradually diminish until at about the end of the fourth month, no protective power apparently remains.

(3) That there is little or no evidence that the case mortality is favourably affected by inoculation. Possibly the fatality of the disease may be reduced for a short period after inoculation.

(4) That there is no evidence to indicate that there is an optimum dose of vaccine to procure the maximum protection, nor whether two prophylactic injections are of more value than a single dose.

(5) That the addition of glucose to the medium for the preparation of the vaccine is associated with no apparent improvement in the results.

(6) That the attack-rate from other respiratory diseases is also favourably influenced by this vaccine; this is probably due to the fact that many of these diseases are causally associated with the pneumococcus.

(7) That other medical diseases are uninfluenced by the prophylactic use of this vaccine.

In addition to organising the prophylactic inoculations against pneumonia Sir Almroth Wright conducted a small number of experiments in regard to the curative value of this vaccine. Alternate cases of pneumonia, occurring amongst the previously vaccinated and the unvaccinated Natives admitted to the W.N.L.A. Hospital, received treatment with vaccine. The hospital case-sheets of the patients thus selected for treatment, as well as those of the controls, were appropriately marked. I have been able to trace 307 such sheets, of which 146 were marked "treated" and 161 "not treated." The correlation obtained between vaccine treatment and recovery is negative in sign, but having regard to its probable error, it is not significant.

TABLE LXIII.

	+	—	Total.	
	Vaccine treatment.			
Recoveries . .	93	112	205	$r_t = -.1059 \pm .0608$
Deaths	53	49	102	$\chi^2 = 0.94150$ $P = .8122$
Total	146	161	307	

These figures can be divided into two sub-groups, (1) Natives who had previously received prophylactic inoculation, and (2) those who

had not. In 110 cases prophylactic inoculation had been given, and in this group the correlation is suggestive that treatment with the vaccine was actually harmful.

TABLE LXIV.

	Vaccine treatment. +	—	Total.	
Recoveries	29	48	77	$r_t = -\cdot3415 \pm \cdot0976$
Deaths	20	13	33	$\chi^2 = 4\cdot37980$ $P = \cdot2274$
Total	49	61	110	

Of the previously uninoculated Natives there is no evidence that vaccine treatment had any effect in influencing the case mortality from this disease; thus,

TABLE LXV.

	Vaccine treatment. +	—	Total.	
Recoveries	64	64	128	$r_t = \cdot0335 \pm \cdot0775$
Deaths	33	36	69	$\chi^2 = 0\cdot08948$ $P = \cdot9822$
Total	97	100	197	

Unfortunately no discoverable system in regard to number of doses, size of dose, or period of the disease at which the doses were given was adopted, so that it is impossible to sub-divide these groups further with any advantage. So far as the results of this therapeutic experiment go we can say that there is no evidence that vaccine treatment was of any value, and in the cases in which a prophylactic dose of vaccine had been previously received there is a certain amount of evidence to suggest that actual harm resulted from such treatment.

I do not think, however, that it would be wise to generalise too much from these results, and probably a large amount of more careful work will have to be done before any final opinion on this question can be satisfactorily arrived at. These results are, nevertheless, somewhat surprising in view of the definite statements which have, from time to time, been made in respect to the efficacy of this particular form of treatment. For example, Dr. J. Eyre[19] writes as follows:—" Turning now to the other phase of bacterial therapy, the employment of ' killed cultivations ' or ' vaccines ' in the treatment of pneumonia, a considerable measure of success is obtained." Also in the same article, after having described the method of dosage with

pneumococcal vaccine, the author proceeds thus:—" By this means the marked rise in available opsonin that naturally precedes the crisis, and the consequent fall in temperature and disappearance of physical signs, may be considerably anti-dated, and the process of cure correspondingly hastened."

The therapeutic inoculations here discussed were carried out entirely under Sir Almroth Wright's personal supervision, and by his assistants, so that it is unlikely that errors in technique can account for the want of success obtained in these cases. No figures are recorded in support of Eyre's assertions, so that it is impossible to hazard any suggestions as to why the results recorded here correspond so badly with those claimed by some European writers.

SECTION V.

General Conclusions.

The conclusions to be drawn from the foregoing investigation may now be briefly stated.

From Section I.:—

(1) That the attack-rate and death-rate from pneumonia amongst "Tropical Natives" living on the Rand are influenced by the country of origin from which the Natives are recruited.

(2) That the case mortality is apparently independent of the country of origin.

(3) That the highest pneumonia attack-rates are found among the gangs which—judged by the number of "detentions" and "rejects"—have the lowest physique.

(4) That the greatest incidence of pneumonia occurs immediately on the arrival of the Natives on the Rand, and decreases as the period of their residence increases.

(5) That the case mortality so far as can be judged from a six-months record is apparently uninfluenced by the length of residence on the Rand.

(6) That pneumonia is most prevalent during the cold season.

From Section II.:—

(7) That the clinical signs and symptoms of pneumonia among "Tropical Natives" do not differ essentially from those among Europeans.

(8) That the length of the febrile period of the disease is probably somewhat shorter among these Natives than among Europeans in the Northern Hemisphere.

(9) That while the methods of the termination of the pyrexial stage are similar to those observed elsewhere, the percentage of cases which terminate by "crisis" is, among these Natives, lower than the percentages recorded for Europeans in other countries.

(10) That there does not appear to be any essential difference in the clinical characters of the attack, nor in the length of the convalescence, whether the fever terminate by "crisis" or by "lysis."

(11) That "second attacks" of pneumonia (as defined in this memoir) are most common immediately after the termination of the first attack, and become progressively more rare as the period after the first increases.

(12) That the rate of "second attacks" among the population of the previously attacked is higher than the primary attack rate among the whole population.

(13) That the case mortality of "second attacks" is probably lower than that of "first attacks."

(14) That pneumococcal meningitis as a complication of pneumonia appears to be considerably more common among "Tropical Natives" than among Europeans.

(15) That amongst the Natives it is more common to find two or more lobes involved in the pneumonic process than amongst Europeans.

(16) That the stage of hepatization of the lung, as observed at the post-mortem, bears no constant relation to the length of the illness before death.

(17) That the most common day for death to take place in pneumonia is the fourth day of illness.

Section III.:—

(18) That there is no evidence from the data examined that pneumonia spreads from case to case.

(19) That the irregular grouping which is observed in some of the tables cannot be satisfactorily accounted for on the assumption that this disease is infectious.

Section IV.:—

(20) That prophylactic inoculation with a pneumococcal vaccine, when employed immediately on the arrival of these Natives on the Rand, appears to reduce the incidence of pneumonia for a limited period.

(21) That the protective value is greatest immediately or shortly after inoculation, and progressively diminishes.

(22) That its effect does not appear to last for more than about four months.

(23) That in these records there is no evidence that prophylactic inoculation has any influence on the case mortality, except possibly for a very short time after the injection.

(24) That the experimental treatment of cases with pneumococcal vaccine did not favourably influence the mortality from the disease.

(25) That there is some evidence to suggest that treatment with pneumococcal vaccine of cases which had recently received a prophylactic injection of a similar vaccine was harmful.

The investigation has thrown but little light on the problem of the causes which determine the outbreak of pneumonia among new arrivals on the Reef; further information is necessary before a satisfactory hypothesis can be propounded. I have recently examined the nasal secretions of a small number of "East Coast" Natives on their arrival on the Rand, with a view to determining whether the pneumococcus is present at this time. The results so far, although a sufficient number have not yet been examined to enable rates to be

satisfactorily determined, show that it is not uncommon to find a pneumococcus in the naso-pharynx of these Natives within a few hours of their arrival in the W.N.L.A. Compound. The obvious question which now requires an answer is—does the pneumococcus exist in the naso-pharynx of these Natives when in their homes, and if not, at what point on their journey to the mines do they become infected?

It has been pointed out in Section I. that the outbreak of pneumonia synchronises with the arrival of the Natives on the Rand, and a similar phenomenon has been observed elsewhere when a change of residence is made. It is of considerable practical importance to determine whether change of climate is accompanied by the appearance of the pneumococcus in a previously uninfected naso-pharynx, or whether there be no increase in the rate of such infections owing to the change in locality. If this latter event were found to be correct, and the increased incidence of pneumonia be not associated with a greater prevalence of the pneumococcus in the throats of the apparently healthy, then the suggestion that the most important factor in determining the occurrence of the disease is some change in the body of the immigrant—such as a lowered resistance—brought about by the alterations in climate would gain support.

There seems little doubt that pneumonia is not unknown, and possibly not even rare, among "Tropical Natives" in their own country. It is notoriously difficult to get reliable information from uneducated Natives. By cross-examining Natives from different parts of the tropical recruiting area, I have obtained an account of a disease which has the symptoms of pain in the chest, spitting of blood-stained sputum, and shortness of breath. This disease is stated to be frequently fatal within three or four days of its onset. Some witnesses state that death may even take place in the first day of illness. Women and children, as well as men, are reported to suffer from it; this is important, as it shows that the disease is not confined to males who have returned from the mines. It is variously stated to be "common" and "not very common" by Natives from different districts. Mozambique and Nyassaland Natives give the native name as "Parare" (literally, pain under the ribs); known by the Maganja da Costa Natives as "Aparu"; by the Yao's as "Chisorma"; Negroes from Blantyre district call it "Kalaso"; B.N.P. name "Chirombo."

These witnesses have uniformly stated, "the disease is a serious one." Although no doubt other diseases may be included under such names, the description given undoubtedly suggests that cases of pneumonia do occur in these districts.

The Secretary of the W.N.L.A. has kindly given me permission to publish the following interesting letter on this subject from Mr. Hanner.

Witwatersrand Native Labour Association, Limited.
Matolla Agency,
18th November, 1913.

The Secretary,
W.N.L.A., Ltd.,
Johannesburg.

Dear Sir,

Re Pneumonia amongst Tropicals.

In reply to yours of the 14th inst. referring to the above, I beg to inform you that a disease very similar to pneumonia is quite common amongst Nyassa and Zambesi Natives.

In Portuguese Nyassaland it is known as "Ngonjua Chifua," or "Kossamolla," and amongst the Natives of the Zambesi valley I believe it is called "Anacoshola Benca," but as it is some sixteen or seventeen years since last I had occasion to speak "Chikunda" I would not be positive as to whether the latter name is correct.

As far as my experience goes I think that chest diseases are more prevalent amongst the Natives of the Upper Lujela (Quilimane district). It may be of interest to you to know that some 25 years ago, when I first went to the Zambesi, they had a tremendous famine which literally wiped out the population by tens of thousands. I myself have seen people lying dead under the trees all over the country, and also in their villages. It became so bad at Tete that they brought the bodies and threw them just outside the cemetery.

The tribe that suffered the most were the people who lived north of Quilimane and towards "Shirwa," and I think that this accounts for the poor physique of the Natives living in this part of the country.

The people who suffer the least from chest diseases are a tribe called "Azimba," who live in a range of mountains some 20 miles north of the "Kebrabassa rapids," and which range runs practically up to "Undis." These people are of a splendid physique, but very wild. When I was living amongst them the paramount Chief was named "Biwi." It may be that these people are not so susceptible to chest complaints, owing to the high altitude at which they live.

Yours faithfully,
(Signed) R. M. HANNER,
Div. Recruiter.

Clearly this hearsay evidence requires confirmation by medical and bacteriological investigation in the districts themselves, not only in regard to the existence of the disease in these parts, but also as to its relative prevalence.

It has already been mentioned that some of the apparent anomalies in regard to the etiology and manifestations of pneumonia may be explicable on the hypothesis that there are several strains or varieties of the pneumococcus with different properties. If this suggestion receives support by subsequent investigation, then the problem of the cause of the prevalence of pneumonia on the Rand will also require investigation from this point of view. It will be necessary to determine whether the type of organism which produces the disease in tropical districts—if the disease does occur there—is the same as that which is common on the Rand, and if not, is the alteration due to a change in type of pneumococcus developed owing to changes in the host, or again, is a freshly acquired organism the cause of the outbreak? If the pneumococcus be found to be much less prevalent in the low-veld than among Natives arriving on the Rand, then it is important to discover at what point in their journey the greater prevalence is acquired. Arrangements are now being made by the Institute to undertake at an early date an investigation on these lines, to examine the Natives at the various compounds on the route of their journey to the Rand, to inspect them in their kraals, and also if possible to study some cases of pneumonia in the native districts.

Since the foregoing sections have been in the press my attention has been drawn to a paper by Drs. Dochez and Gilespie[27] on the differentiation of strains of pneumococci. In view of this work, and of its confirmation by Dr. Lister in this Institute from a somewhat different standpoint, it will be important to discover whether "second attacks" of pneumonia are due to a different strain of organism. If, as seems possible, this may be so, it will be necessary to determine whether the "second attack" is a new infection, a latent infection becoming virulent owing to the effects of the previous illness, or a variation of the original strain arising by selection. Another problem of importance is to determine whether the pneumococcus found in normal throats is the source of infection to its bearer who develops pneumonia, and if so, whether any discoverable change in its type precedes the attack.

The present investigation will, I hope, serve a useful purpose by having established certain facts in the etiology of this disease among "Tropical Natives," and by having directed attention to some of the lacunae in our knowledge. Continued scientific investigation is required before any further advance in the production of an artificial immunity against attack, or any solution to the problem of increasing the resistance of the attacked, can be expected. The wholesale application of empiric remedies cannot but have a reactionary effect, and the tendency in some quarters on the Reef to consider the problem of pneumonia as settled will only result in retarding progress.

In conclusion I would like to take this opportunity of thanking Mr. B. G. Lloyd, General Manager of the Witwatersrand Native Labour Association, for his courtesy in giving me free access to the returns of the Association, and Dr. G. A. Turner, Medical Officer to the Association, for a similar favour in connection with the hospital reports and records, without which this investigation could never have been undertaken. My thanks are also due to Dr. J. McD. Troup for much asisstance, particularly in regard to some of the mathematical problems in Section III.; and to Dr. W. Watkins-Pitchford, the Director of the Institute, for constant help and advice in the numerous discussions which arose in the course of the investigation, and for his kindness in reading through the manuscript. I would also take this opportunity to express my appreciation of the painstaking and conscientious manner in which Mr. P. Hardwick has carried out the laborious duty of compiling and checking the returns of the inoculation experiments.

Finally, I wish to state, that although I have sometimes had occasion to adversely criticise statements made in the leading text-books of Medicine, yet this has not been done in a captious spirit; but because such criticisms were essential in order to show that the statements had not been disregarded. No thoughtful practitioner can be insensible of the debt the profession owes to the authors of these books, nor can he fail to realise the difficulty of maintaining every portion of such works at a uniformly high level.

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APPENDIX.

A Pneumococcal Vaccine Inoculation Experiment on Natives in the employ of the Central Mining and Rand Mines Groups.

Since the manuscript of the foregoing sections has been in the press the following tables, summarising the results obtained from an experiment in anti-pneumonia vaccination, have been issued. This experiment has been carried out on the mines under the control of the Central Mining and Rand Mines Groups, and my thanks are due to the managements for permission to publish the returns.

In consultation with the Medical Officers of these mines it was decided to inoculate three-quarters of all "Tropical," "East Coast," and Basuto Natives on the properties, the remaining quarter to be left as controls to the larger group. Before being inoculated the Natives were grouped according to the length of their previous service on the mine, and every fourth "boy" excluded from the inoculation, and classed as a control. Only one inoculation was given, the dose being "1000 millions." The results of this experiment, for the first four months, are reproduced in Tables A., B., C., and D.

It is unnecessary to lay stress on the fact that in an investigation such as the present, where many managements are concerned in carrying out a somewhat complicated procedure, the experimental errors will necessarily tend to be larger than when the whole experiment is conducted by one set of persons. It would appear, however, that on many of the mines, and of these some are amongst the largest employers of native labour in the groups, the greatest care was taken to carry out minutely the instructions issued, while it is equally certain that on others much less trouble was taken.

On one large group of mines the Medical Officers declined to be informed as to whether a patient was or was not an inoculated "boy" until he left hospital, in order that no unconscious bias should affect their diagnosis. This is an example of a detail in method which might with advantage be followed by all medical men who desire to obtain a true estimate of the facts of such experiments. No very extensive knowledge of human nature is required to establish that the presence of a bias, even amongst those who are most desirous of remaining strictly impartial, is inevitable where an observer has any opinion as to the efficacy or otherwise of any procedure the effect of which he is investigating. Further, no one who has taken an interest in a subject and heard it discussed, can but have formed some mental estimate of its value, however tentatively such an estimate may be held. This digression has been made, because the effect of an unconscious bias

does not appear to be sufficiently recognised, and, further, because some of the reports received would have had a considerably increased value had the method adopted by these Medical Officers been followed by their authors.

Nevertheless, so far as can be ascertained there does not appear to be any special source of error in regard to this experiment which would apply to the inoculated more than to the "controls," or *vice versa*.

Turning to an examination of the data given in Tables A, B, C, D, appearing at end of text, the effect of inoculation may be studied by the same methods as used in Section IV.; but here the calculation of the correlation from the "four-fold table" will not lead to any useful purpose. It will be sufficient to calculate the "goodness of fit" of observation to theory on the basis of independence. The following four tables are constructed from the group "all Natives" for each of the four months, and deal with the number attacked among the "inoculated" and the "controls."

TABLE F. (First Month).

	Inoculation. +	—	Total.	
Not attacked ...	15618	5183	20801	$\chi^2 = 0{\cdot}047876$
Attacked	83	29	112	$P = {\cdot}9905$
Total	15701	5212	20913	

TABLE G. (Second Month).

	Inoculation. +	—	Total.	
Not attacked ...	14883	4900	19783	$\chi^2 = 0{\cdot}149388$
Attacked	55	16	71	$P = {\cdot}9703$
Total	14938	4916	19854	

TABLE H. (Third Month).

	Inoculation. +	—	Total.	
Not attacked ...	13883	4577	18460	$\chi^2 = 4{\cdot}346127$
Attacked	39	22	61	$P = {\cdot}2304$
Total	13922	4599	18521	

TABLE I. (FOURTH MONTH).

	Inoculation. +	—	Total.	
Not attacked ...	12980	4314	17294	$\chi^2 = 3{\cdot}080320$
Attacked	38	6	44	$P = {\cdot}3812$
Total	13018	4320	17338	

In the first month the attack-rates were actually slightly in favour of the "controls," and in the second in favour of the "inoculated," but clearly neither value has any significance. In the third month the balance in favour of the inoculated is more marked, and the chance against a divergence as great as that observed having arisen from errors of sampling is one to four. In the fourth month the lower attack-rate is again found amongst the uninoculated.

Averaging the population for the four months, the following table is obtained:—

TABLE J.

	Inoculation. +	—	Total.	
Not attacked ...	14180	4689	18869	$\chi^2 = 0{\cdot}018802$
Attacked	215	73	288	$P = {\cdot}9963$
Total	14395	4762	19157	

Thus from these returns, employing the group "All Natives" as the measure of efficacy, no support is obtained for the hypothesis that the pneumococcal vaccination had any effect in lowering the attack-rate from pneumonia. Can we therefore *legitimately assert* that these figures prove that the prophylactic use of this vaccine is without value? I think not. The attack-rates from this disease were comparatively speaking low throughout the period, and, therefore, a real protection might be masked by the errors of sampling. The probability of such an occurrence can be investigated in the following way. We may assume that a certain percentage reduction in the attack-rate should follow inoculation, and then by regarding the attack-rate of either group as representing the normal for that group we can calculate the rate to be expected in the other group, and from it the most probable number of Natives who would, on this hypothesis, have contracted the disease. The distribution thus found is then regarded as the "most probable," and the deviation from it as being due to errors of sampling. In the following tables I have regarded the observed rate of each group in turn as the "normal": the true normal rate would probably lie between these two values, and therefore the value of P should lie between the values as given in the tables.

TABLE K. (First Month).

Basis of 30 per cent. reduction due to Inoculation.

Normal rate 5·6 per thousand.
∴ rate among Inoculated 3·9 per thousand.
Distribution expected.

+	−
15640	5183
81	29

$\chi^2 = 7{\cdot}9652$
$P = {\cdot}0469$

Normal rate 5·3 per thousand.
∴ rate among "Controls" 7·6 per thousand.
Distribution expected.

+	−
15618	5172
83	40

$\chi^2 = 3{\cdot}0484$
$P = {\cdot}3853$

TABLE L. (Second Month).

Basis of 20 per cent. reduction due to Inoculation.

Normal rate 3·3 per thousand.
∴ rate among Inoculated 2·6 per thousand.
Distribution expected.

+	−
14899	4900
39	16

$\chi^2 = 7{\cdot}546618$
$P = {\cdot}0577$

Normal rate 3·7 per thousand.
∴ rate among "Controls" 4·6 per thousand.
Distribution expected.

+	−
14883	4893
55	23

$\chi^2 = 2{\cdot}140451$
$P = {\cdot}5470$

TABLE M. (Third Month).

Basis of 10 per cent. reduction due to Inoculation.

Normal rate 4·8 per thousand.
∴ rate among Inoculated 4·3 per thousand.
Distribution expected.

+	−
13862	4577
62	22

$\chi^2 = 7{\cdot}381818$
$P = {\cdot}0620$

Normal rate 2·8 per thousand.
∴ rate among "Controls" 3·1 per thousand.
Distribution expected.

+	−
13883	4585
39	14

$\chi^2 = 4{\cdot}585386$
$P = {\cdot}2090$

TABLE N. (Total for Four Months).

Basis of 15 per cent. reduction due to Inoculation.

Normal rate 15·3 per thousand.
∴ rate among Inoculated 13·0 per thousand.
Distribution expected.

+	−
14198	4689
187	73

$\chi^2 = 4{\cdot}247731$
$P = {\cdot}2393$

Normal rate 14·9 per thousand.
∴ rate among "Controls" 17·5 per thousand.
Distribution expected.

+	−
14180	4679
215	83

$\chi^2 = 1{\cdot}226191$
$P = {\cdot}7495$

This method of considering the problem will only give us a rough guide to the improbability that the observed results have arisen from a population which, if large enough to render the errors of sampling negligible, would have shown the percentage reduction assumed in the tables. The right hand table of each pair is based on the more reasonable assumption, because the rates calculated from the larger group will be less influenced by errors of sampling; clearly, however, neither table is more than a rough approximation, as the rate in the group called "normal" is considered as being free from errors of sampling. The results, nevertheless, seem to me to indicate that we cannot safely conclude that these returns *prove* pneumococcal vaccination, even in this experiment, to be valueless; for variations such as those observed might have arisen from errors of sampling, even if the percentage reductions such as those assumed were to have been expected. It will be necessary, therefore, to wait for larger samples —when such small total rates occur amongst the attacked—before dogmatising on this matter, and I do not think this experiment should be accepted as definitely negativing the results found from the "8000 experiment." All we can assert is, that *this experiment*, carried out on the mines among a group of Natives heterogeneous as to race and length of service, do not confirm the results deduced from the "8000 experiment." We can, however, from the tables supplied, get rid of these sources of heterogenity, and the following tables deal with the results for the four months for each territorial group. Further sub-division has not been undertaken owing to the small number of cases which occur in sub-groups, except in the case of the group "All Natives," where the values of χ^2 and P are shown for the sub-groups according to period of service.

TABLE O.

Basutos (four months).

	Inoculation. +	—	Total.	
Not attacked ...	923	305	1228	$\chi^2 = 0{\cdot}000000$
Attacked	15	5	20	$P = 1{\cdot}$
Total	938	310	1248	

TABLE P.

"East Coast" (four months).

	Inoculation. +	—	Total.	
Not attacked ...	9612	3213	12825	$\chi^2 = 0{\cdot}348257$
Attacked	100	38	138	$P = {\cdot}9308$
Total	9712	3251	12963	

TABLE Q.

"Tropicals" (four months).

	Inoculation. +	—	Total.	
Not attacked ...	3645	1172	4817	$\chi^2 = 0{\cdot}174364$
Attacked	100	29	129	$P = {\cdot}9653$
Total	3745	1201	4946	

The tables of the sub-groups of the group "All Natives," classed according to period of service on the mines before inoculation, are as under:—

TABLE R.

Under three months' service.

	Inoculation. +	—	Total.	
Not attacked ...	3901	1220	5121	$\chi^2 = 0{\cdot}046428$
Attacked	93	28	121	$P = {\cdot}9908$
Total	3994	1248	5242	

TABLE S.

Under six months' service.

	Inoculation. +	—	Total.	
Not attacked ...	4138	1357	5495	$\chi^2 = 1{\cdot}479008$
Attacked	63	28	91	$P = {\cdot}6916$
Total	4201	1385	5586	

TABLE T.

Under nine months' service.

	Inoculation. +	—	Total.	
Not attacked ...	2683	866	3549	$\chi^2 = 0{\cdot}428097$
Attacked	37	11	48	$P = {\cdot}9149$
Total	2720	877	3597	

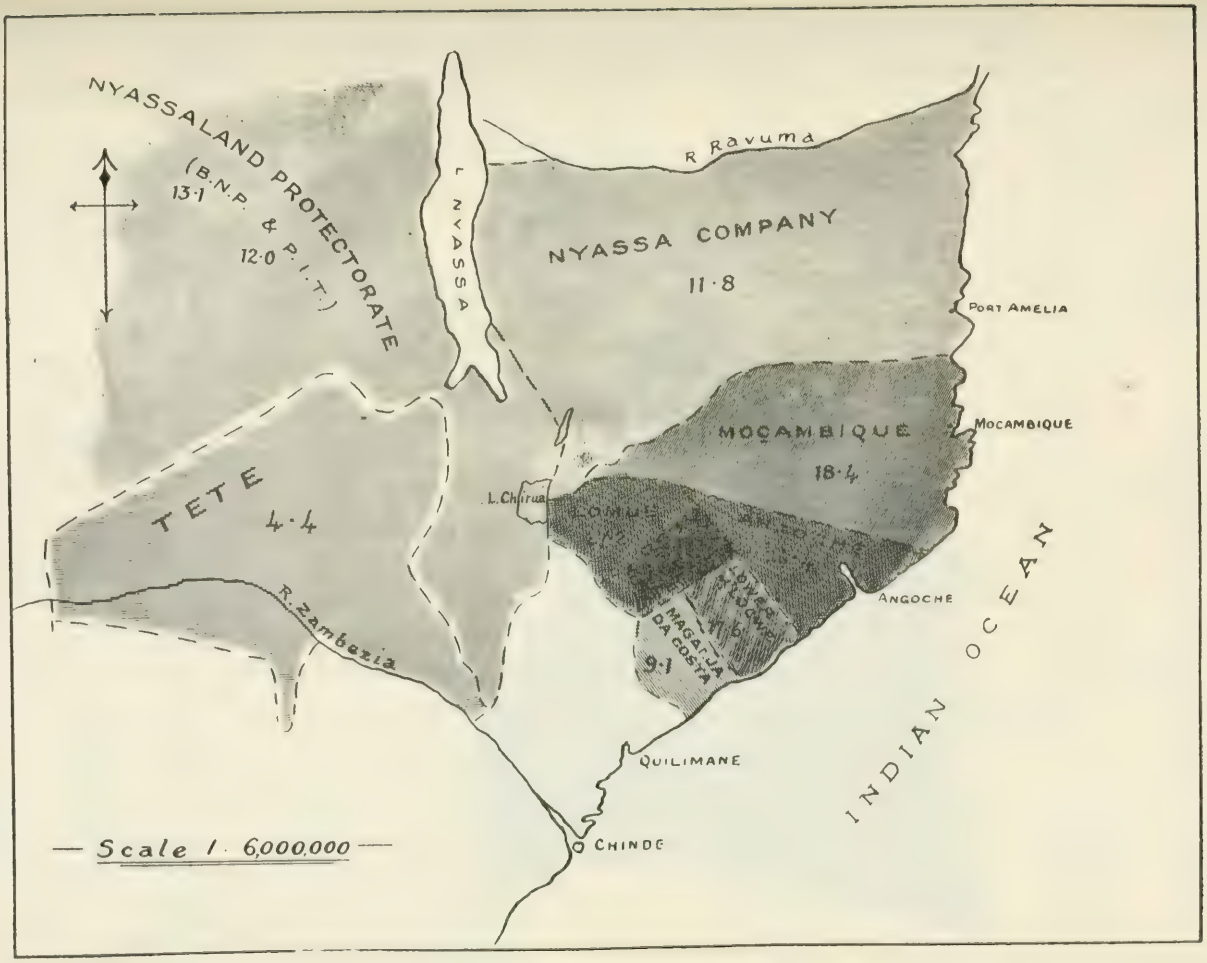
TABLE U.

Over nine months' service.

	Inoculation. +	—	Total.	
Not attacked ...	3457	1246	4703	$\chi^2 = 0{\cdot}191569$
Attacked	22	6	28	$P = {\cdot}9619$
Total	3479	1252	4731	

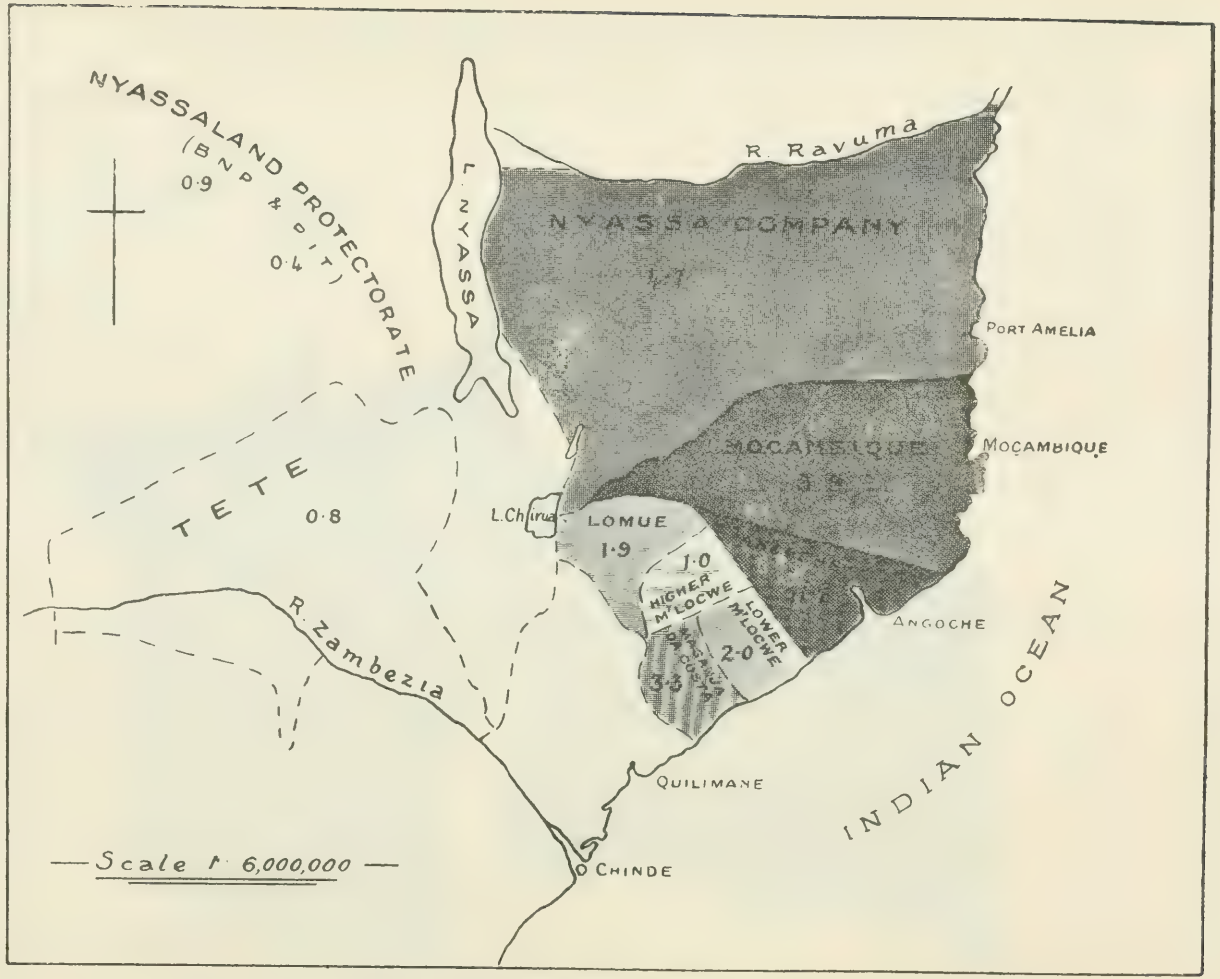
It is evident, therefore, that the absence of any indication of the efficacy of vaccination as a prophylactic measure is not due to the masking of the real effect due to the heterogeneity of the group "All Natives." In none of the sub-groups examined in Tables O. to U. is there any evidence that the inoculated Natives had an appreciably lower attack-rate than the "controls."

Finally, it may be remarked that these tables illustrate once more the fall in the attack-rate with increase in the period of residence on the Reef. The total attack-rate for the four months for Natives, under three months is 23 per thousand, under six months 16 per thousand, under nine months 13 per thousand, and over nine months 6 per thousand.



SKETCH MAP I.

Showing recruiting areas north of Lat. 22° S. of W.N.L.A., Ltd. Shaded to indicate comparative death-rates on the Rand from Pneumonia.



Showing recruiting areas north of Lat. 22° S. of W.N.L.A., Ltd. Shaded to indicate comparative death-rates on the Rand from Cerebro-spinal Meningitis.

A

CENTRAL MINING—RAND MINES GROUP.

PNEUMOCOCCIC VACCINE INOCULATION EXPERIMENT ON NATIVES.

AMENDED RETURN FOR FIRST MONTH AFTER INOCULATION.

Class of Native.	INOCULATED NATIVES.										CONTROL NATIVES.									
	No. of Natives originally inoculated.	Average No. of inoculated Natives in Co's service during 1st month succeeding inoc'n.	PNEUMONIA.				OTHER RESPIRATORY DISEASES.				No. of Natives originally set aside as controls.	Average No. of Control Natives in Co's service during 1st month.	PNEUMONIA.				OTHER RESPIRATORY DISEASES.			
			ADMISSIONS TO HOSPITAL.		DEATHS		ADMISSIONS TO HOSPITAL.		DEATHS.				ADMISSIONS TO HOSPITAL.		DEATHS.		ADMISSIONS TO HOSPITAL.		DEATHS.	
			No.	Rate per 1,000 per month	No.	Rate per 1,000 per month.	No.	Rate per 1,000 per month	No.	Rate per 1,000 per month			No.	Rate per 1,000 per month	No.	Rate per 1,000 per month	No.	Rate per 1,000 per month	No.	Rate p r 1,000 per month
EAST COAST.—																				
Under 3 months' service	2,279	2,105	11	5·2	3	1·4	10	4·8			700	639	3	4·7			4	6·3		
From 3—6 ,, ,,	2,869	2,736	6	2·2			7	2·6			988	933	3	3 2	1	1·1	8	8·6		
,, 6—9 ,, ,,	2,199	2,088	6	2·9	1	·5	2	1·0			732	693	6	8·7			1	1·4		
Over 9 ,, ,,	3,848	3,413	7	2·1	2	·6	2	·6			1,369	1,220					1	·8		
Totals and Averages	11,195	10,342	30	2·9	6	·6	21	2·0			3,789	3,485	12	3 4	1	·3	14	4·0		
TROPICALS.—																				
Under 3 months' service	1,516	1,454	22	15·1	4	2 8	7	4·8			471	456	6	13·2	2	4·4	2	4·4		
From 3—6 ,, ,,	1,535	1,471	18	12·2	4	2·7	7	4·8			494	485	6	12·4			1	2·1		
,, 6—9 ,, ,,	804	768	6	7·8	1	1·3	4	5·2			261	248	1	4·0			4	16·1	1	4·0
Over 9 ,, ,,	559	402					1	2·5			169	130								
Totals and averages	4,414	4,095	46	11·2	9	2·2	19	4 6			1,395	1,319	13	9·9	2	1·5	7	5·3	1	·8
BASUTOS.—																				
Under 3 months' service	915	786	7	8·9			1	1·3			285	248	4	16·1			1	4·0		
From 3—6 ,, ,,	341	268									118	91								
,, 6—9 ,, ,,	90	48									27	14								
Over 9 ,, ,,	190	162									63	55								
Totals and Averages	1,536	1,264	7	5·5			1	·8			493	408	4	9·8			1	2·5		
ALL NATIVES —																				
Under 3 months' service	4,710	4,345	40	9·2	7	1·6	18	4·1			1,456	1,343	13	9·7	2	1·5	7	5·2		
From 3—6 ,, ,,	4,745	4,475	24	5·4	4	·9	14	3·1			1,600	1,509	9	5·6	1	·6	9	6·0		
,, 6—9 ,, ,,	3,093	2,904	12	4·1	2	·7	6	2·1			1,020	955	7	7·3			5	5·2	1	1·0
Over 9 ,, ,,	4,597	3,977	7	1 8	2	·5	3	·8			1,601	1,405					1	·7		
Grand Totals and Averages	17,145	15,701	83	5·3	15	1·0	41	2·6			5,677	5,212	29	5·6	3	·6	22	4·2	1	·2

CENTRAL MINING—RAND MINES GROUP.

PNEUMOCOCCIC VACCINE INOCULATION EXPERIMENT ON NATIVES.

RETURN FOR SECOND MONTH AFTER INOCULATION.

Class of Native.	INOCULATED NATIVES.									CONTROL NATIVES.								
	No. of Natives originally inoculated.	Average No. of inoculated Natives in Co's service during 2nd month succeeding inocn.	PNEUMONIA.				OTHER RESPIRATORY DISEASES.				No. of Natives originally set aside as controls.	Average No. of Control Natives in Co's service during 2nd month.	PNEUMONIA.				OTHER RESPIRATORY DISEASES.			
			ADMISSIONS TO HOSPITAL.		DEATHS		ADMISSIONS TO HOSPITAL.		DEATHS.				ADMISSIONS TO HOSPITAL.		DEATHS.		ADMISSIONS TO HOSPITAL.		DEATHS.	
			No.	Rate per 1,000 per month.	No.	Rate per 1,000 per month.	No.	Rate per 1,000 per month	No.	Rate per 1,000 per month			No	Rate per 1,000 per month	No.	Rate per 1,000 per month	No.	Rate per 1,000 per month	No.	Rate per 1,000 per month
EAST COAST.																				
Under 3 months' service	2,279	2,073	9	4·3			9	4·3	1	·5	700	636	1	1·6			2	3·1		
From 3–6 ,, ,,	2,869	2,740	8	2·9	2	·7	7	2·6			988	904	5	5·5	1	1·1	4	4·4	1	1·1
,, 6–9 ,, ,,	2,199	2,111	4	1·9	1	·5	4	1·9	1	·5	732	678								
Over 9 ,, ,,	3,848	3,157	7	2·2			7	2·2	2	·6	1,369	1,137	2	1·8	1	·9	2	1·8		
Totals and Averages	11,195	10,081	28	2·8	3	·3	27	2·7	4	·4	3,789	3,355	8	2·4	2	·6	8	2·4	1	·3
TROPICALS.—																				
Under 3 months' service	1,516	1,377	7	5·1	1	7	5	3·6			471	438	2	4·6						
From 3–6 ,, ,,	1,535	1,349	7	5·2			4	3·0			474	438	3	6·8	1	2·3				
,, 6–9 ,, ,,	804	745	6	8·0	2	2·7					261	237	1	4·2						
Over 9 ,, ,,	559	306	1	3·3			1	3·3			169	102								
Totals and averages	4,414	3,777	21	5·6	3	·8	10	2·6			1,395	1,215	6	4·9	1	·8				
BASUTOS.—																				
Under 3 months' service	915	709	4	5·6	2	2·8	1	1·4			285	220	1	4·5	1	4·5	1	4·5		
From 3–6 ,, ,,	341	198									118	71								
,, 6–9 ,, ,,	90	39									27	11								
Over 9 ,, ,,	190	134	2	14·9							63	44								
Totals and Averages	1,536	1,080	6	5·6	2	1·9	1	·9			493	346	1	2·9	1	2·9	1	2·9		
ALL NATIVES—																				
Under 3 months' service	4,710	4,159	20	4.8	3	·7	15	3·6	1	·2	1,456	1,294	5	3·9	1	·8	3	2·3		
From 3–6 ,, ,,	4,745	4,287	15	3·5	2	·5	11	2·6			1,600	1,413	8	5·7	2	1·4	4	2·8	1	·7
,, 6–9 ,, ,,	3,093	2,895	10	3·4	3	1·0	4	1·4	1	·3	1,020	926	1	1·1						
Over 9 ,, ,,	4,597	3,597	10	2·8			8	2·2	2	·6	1,601	1,283	2	1·6	1	·8	2	1·6		
Grand Totals and Averages	17,145	14,938	55	3·7	8	·5	38	2·5	4	·3	5,677	4,916	16	3·3	4	·8	9	1·8	1	·2

C

CENTRAL MINING—RAND MINES GROUP.

PNEUMOCOCCIC VACCINE INOCULATION EXPERIMENT ON NATIVES.

RETURN FOR THIRD MONTH AFTER INOCULATION

Class of Native.	INOCULATED NATIVES.										CONTROL NATIVES.									
	No. of Natives originally inoculated	Average No. of inoculated Natives in Co.'s service during 3rd month succeeding inocn.	PNEUMONIA.				OTHER RESPIRATORY DISEASES.				No. of Natives originally set aside as controls.	Average No. of Control Natives in Co.'s service during 3rd month.	PNEUMONIA.				OTHER RESPIRATORY DISEASES.			
			ADMISSIONS TO HOSPITAL.		DEATHS.		ADMISSIONS TO HOSPITAL.		DEATHS.				ADMISSIONS TO HOSPITAL.		DEATHS.		ADMISSIONS TO HOSPITAL.		DEATHS.	
			No.	Rate per 1,000 per month	No.	Rate per 1,000 per month	No.	Rate per 1,000 per month	No.	Rate per 1,000 per month			No.	Rate per 1,000 per month	No.	Rate per 1,000 per month	No.	Rate per 1,000 per month	No.	Rate per 1,000 per month
EAST COAST.																				
Under 3 months' service	2,279	1,976	8	4·0	1	0·5	5	2·5	1	0·5	700	611	5	8·2			2	3·3		
From 3 6 ,, ,,	2,869	2,660	7	2·6	2	0·8	7	2·6	1	0·4	988	871	5	5·7			2	2·3		
,, 6 9 ,, ,,	2,199	2,014	4	2·0	1	0·5	6	3.0	1	0·5	732	645	1	1·6	1	1·6	1	1·6		
Over 9 ,, ,,	3,848	2,848	4	1·4	1	0·4	3	1·1	1	0·4	1,369	1,045	3	2·9						
Totals and Averages	11,195	9,498	23	2·4	5	0·5	21	2·2	4	0·4	3,789	3,172	14	4·4	1	0·3	5	1·6		
TROPICALS.																				
Under 3 months' service	1,516	1,315	7	5·2	1	0 7	1	0·7			471	432	5	11 6						
From 3 6 ,, ,,	1,535	1,306	4	3·1			2	1·5			494	422	3	7·1	1	2·4	1	2·4		
,, 6 9 ,, ,,	804	708	4	5·6	1	1·4					261	220								
Over 9 ,, ,,	559	257					1	3 9			169	84					1	11 9		
Totals and averages	4,414	3,616	15	4·1	2	0·6	4	1·1			1,395	1,158	8	6·9	1	0·9	2	1.7		
BASUTOS.																				
Under 3 months' service	915	566	1	1·8			1	1·8			285	177								
From 3 6 ,, ,,	341	130									118	46								
,, 6 9 ,, ,,	90	21									27	10								
Over 9 ,, ,,	190	91									63	36								
Totals and Averages	1,536	808	1	1·2			1	1·2			493	269								
ALL NATIVES																				
Under 3 months' service	4,710	3,887	16	4.1	2	0 5	7	1·8	1	0·3	1,456	1,220	10	8 2			2	1·6		
From 3 6 ,, ,,	4,745	4,096	11	2·7	2	0·5	9	2 2	1	0·2	1,600	1,339	8	6·0	1	0·7	3	2·2		
,, 6 9 ,, ,,	3,093	2,743	8	2 9	2	0·7	6	2·2	1	0·4	1,020	875	1	1 1	1	1·1	1	1·1		
Over 9 ,, ,,	4,597	3,196	4	1 3	1	0.3	4	1·3	1	0·3	1,601	1,165	3	2·6			1	0·9		
Grand Totals and Averages	17,145	13,922	39	2·8	7	0·5	26	1·9	4	0·3	5,677	4,599	22	4·8	2	0 4	7	1·5		

D

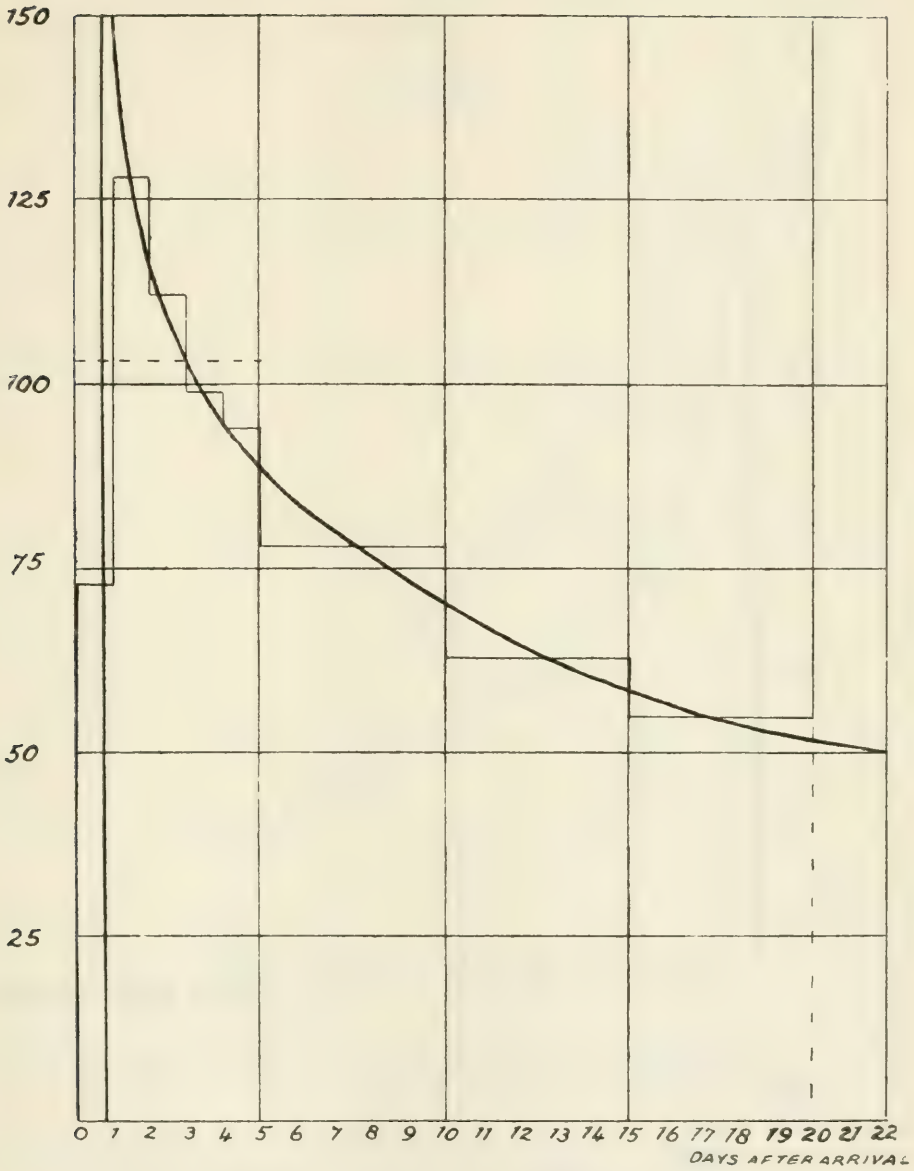
CENTRAL MINING—RAND MINES GROUP.

PNEUMOCOCCIC VACCINE INOCULATION EXPERIMENT ON NATIVES.

RETURN FOR FOURTH MONTH AFTER INOCULATION

Class of Native.	INOCULATED NATIVES.										CONTROL NATIVES.									
	No. of Natives originally inoculated	Average No. of inoculated Natives in Co.'s service during 4th month succeeding inocn.	PNEUMONIA.				OTHER RESPIRATORY DISEASES				No. of Natives originally set aside as controls.	Average No. of Control Natives in Co.'s service during 4th month.	PNEUMONIA.				OTHER RESPIRATORY DISEASES.			
			ADMISSIONS TO HOSPITAL.		DEATHS		ADMISSIONS TO HOSPITAL.		DEATHS.				ADMISSIONS TO HOSPITAL.		DEATHS.		ADMISSIONS TO HOSPITAL.		DEATHS.	
			No.	Rate per 1,000 per month	No.	Rate per 1,000 per month	No.	Rate per 1,000 per month	No.	Rate per 1,000 per month			No.	Rate per 1,000 per month	No.	Rate per 1,000 per month	No.	Rate per 1,000 per month	No.	Rate per 1,000 per month
EAST COAST.																				
Under 3 months' service	2,279	1,876	10	5·3	1	0·5	3	1·6			700	582					1	1·7		
From 3 6 ,, ,,	2,869	2,572	5	1·9	1	0 4	9	3·5			988	848	2	2·4			1	1·2	1	1·2
,, 6 9 ,, ,,	2,199	1,726	3	1·7	1	0.6	7	4.1			732	540	1	1·9	1	1·9				
Over 9 ,, ,,	3,848	2,755	1	0·4	1	0·4	3	1·1	1	0·4	1,369	1,021	1	1·0			3	2·9		
Totals and Averages	11,195	8,929	19	2·1	4	0·4	22	2·5	1	0·1	3,789	2,991	4	1 3	1	0·3	5	1·7	1	0·3
TROPICALS.—																				
Under 3 months' service	1,516	1,318	7	5·3			7	5·3			471	421					1	2·4		
From 3 6 ,, ,,	1,535	1,272	7	5·5	1	0 8	5	3·9			494	400	1	2 5			1	2·5		
,, 6 9 ,, ,,	804	566	4	7·1	2	3·5	2	3 5			261	191	1	5 2						
Over 9 ,, ,,	559	334									169	102								
Totals and averages	4,414	3,490	18	5·2	3	0·9	14	4 0			1,395	1,114	2	1·8			2	1.8		
BASUTOS.—																				
Under 3 months' service	915	391									285	131								
From 3 6 ,, ,,	341	102	1	9·8							118	31								
,, 6—9 ,, ,,	90	48									27	19								
Over 9 ,, ,,	190	58									63	34					1	29·4		
Totals and Averages	1,536	599	1	1·7							493	215					1	4·7		
ALL NATIVES—																				
Under 3 months' service	4,710	3,585	17	4.7	1	0 3	10	2·8			1,456	1,134					2	1·8		
From 3—6 ,, ,,	4,745	3,946	13	3·3	2	0·5	14	3 5			1,600	1,279	3	2 3			2	1·6	1	0·8
,, 6—9 ,, ,,	3,093	2,340	7	3 0	3	1·3	9	3·8			1,020	750	2	2·7	1	1·3				
Over 9 ,, ,,	4,597	3,147	1	0 3	1	0.3	3	1·0	1	0·3	1,601	1,157	1	0·9			4	3·5		
Grand Totals and Averages	17,145	13,018	38	2·9	7	0·5	36	2·8	1	0·1	5,677	4,320	6	1·4	1	0·2	8	1·9	1	0·2

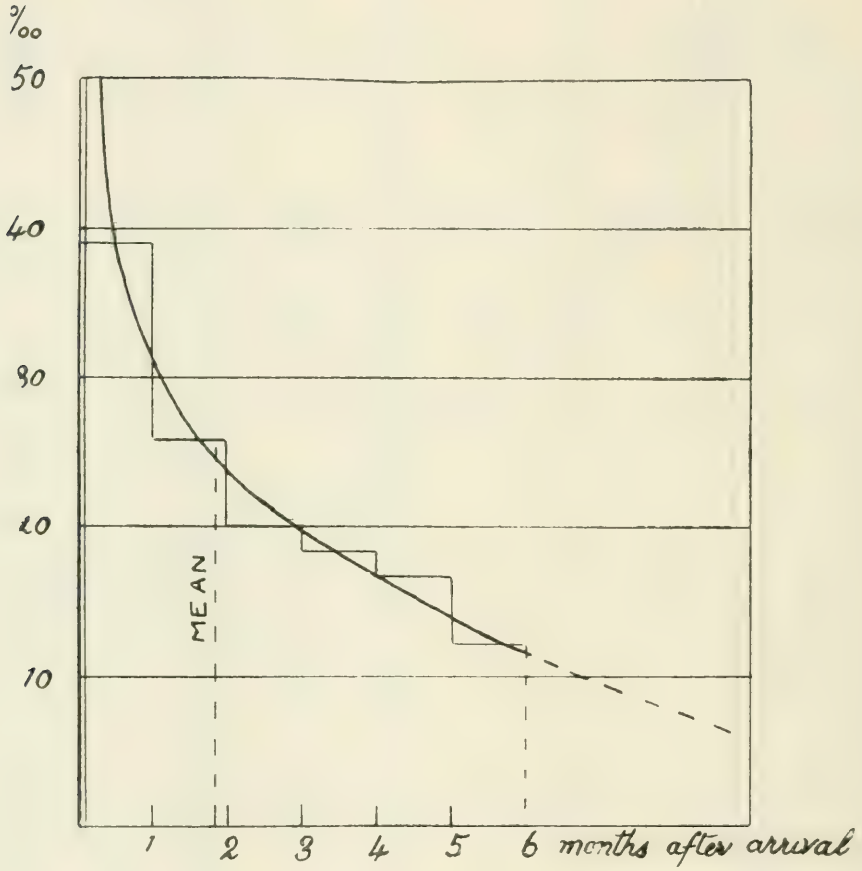
CHART I.



Pneumonia attacks. W.N.L.A. Compound. Tropical Natives. According to number of days after arrival in compound from homes. Population constants for first 21 days.

$$y = 66{\cdot}74 \left(\frac{x}{4{\cdot}9276} - 1 \right)^{-.2513} \left(1 - \frac{x}{26{\cdot}0598} \right)^{1{\cdot}3290} + 50$$

CHART II.



Pneumonia attack-rate per 1,000. For months after arrival on Rand. "Tropical" Natives. [8,123.]

$$y = 213{\cdot}3\left(\frac{x}{3{\cdot}8204} - 1\right)^{-{\cdot}3012}\left(1 - \frac{x}{9{\cdot}9822}\right)^{{\cdot}7870} + 10$$

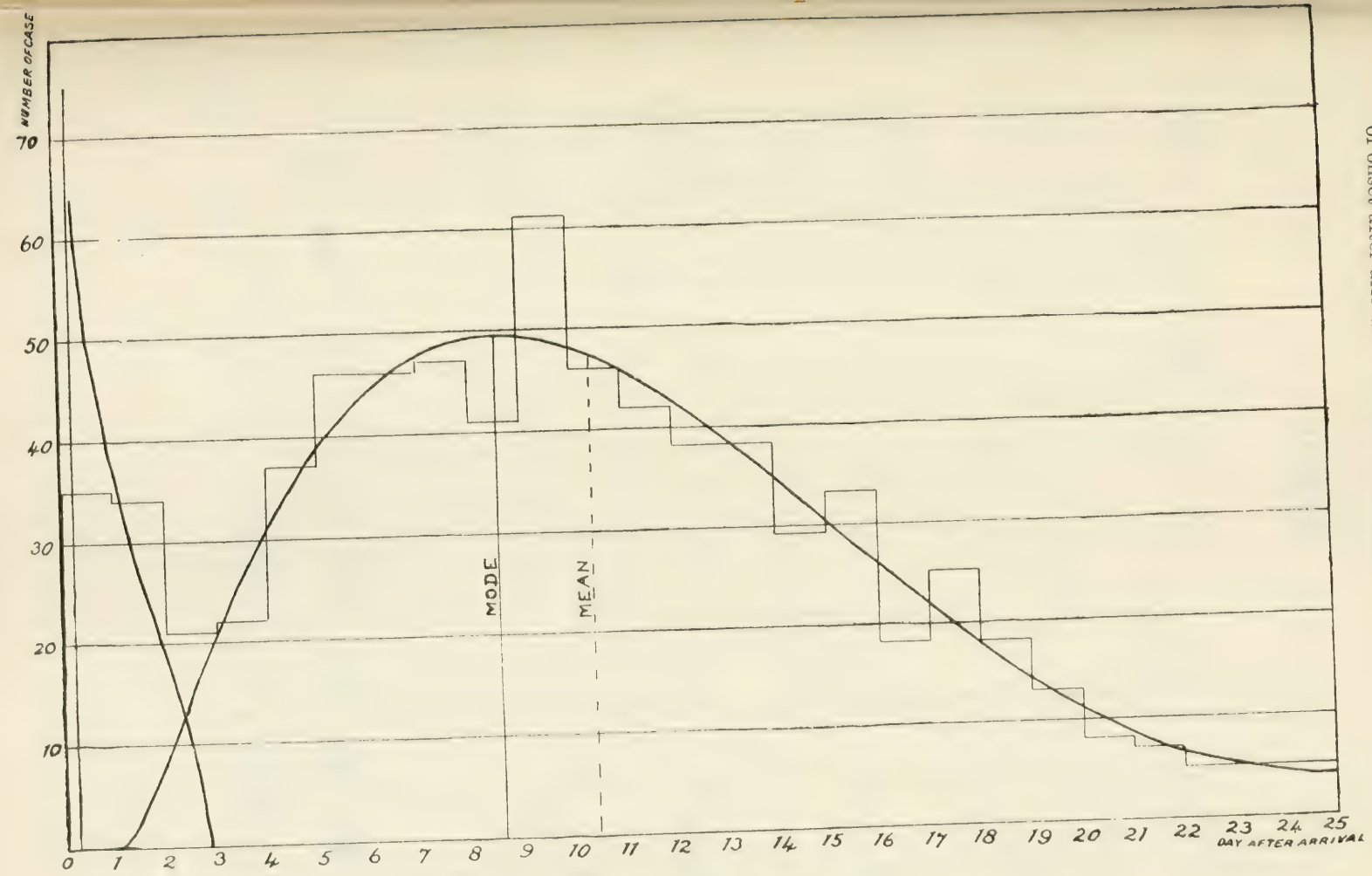


CHART III.

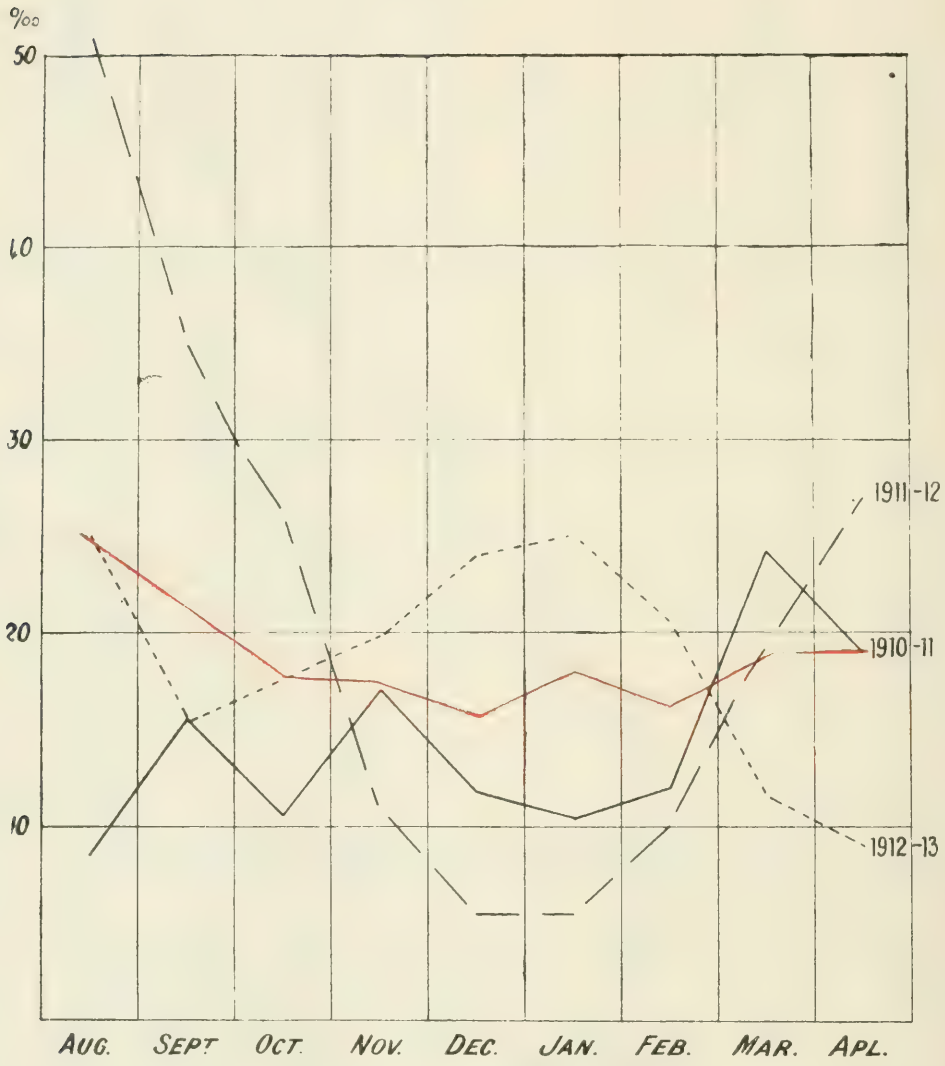
Cerebro-spinal Meningitis. Distribution of cases according to day of onset after arrival in W.N.L.A. Compound.

First Curve $y = 54{\cdot}79\left(\frac{x}{{\cdot}7662} - 1\right)^{-{\cdot}1574}\left(1 - \frac{x}{3{\cdot}3791}\right)^{{\cdot}6942}$ $N = 70 = 9{\cdot}8\%$

Second Curve $y = 45{\cdot}43\left(1 + \frac{x}{6{\cdot}8588}\right)^{1{\cdot}0758}\left(1 - \frac{x}{17{\cdot}6066}\right)^{2{\cdot}7616}$ $N = 650 = 90{\cdot}2\%$

$N = 720.$

CHART IV.



Death-rate from pneumonia for months August--April.
Red line, average of three years.

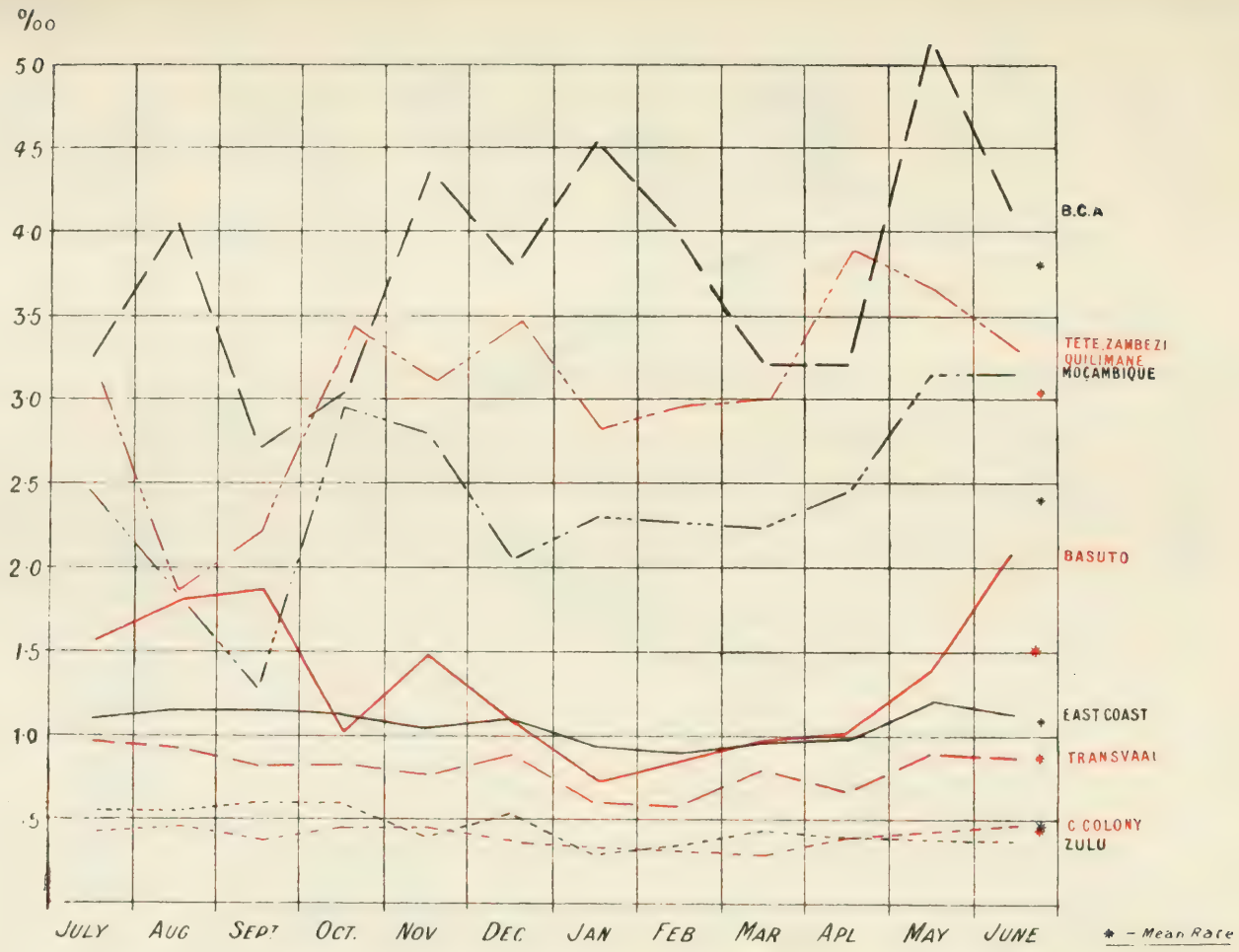
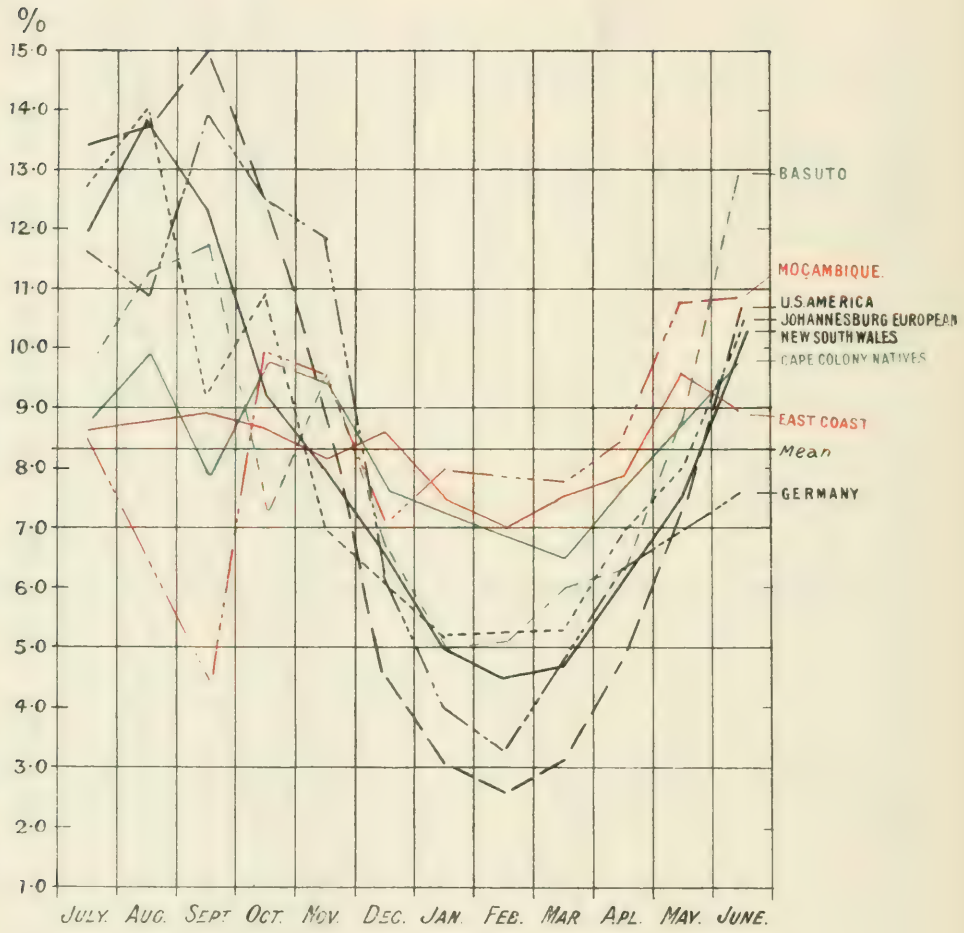


CHART V.

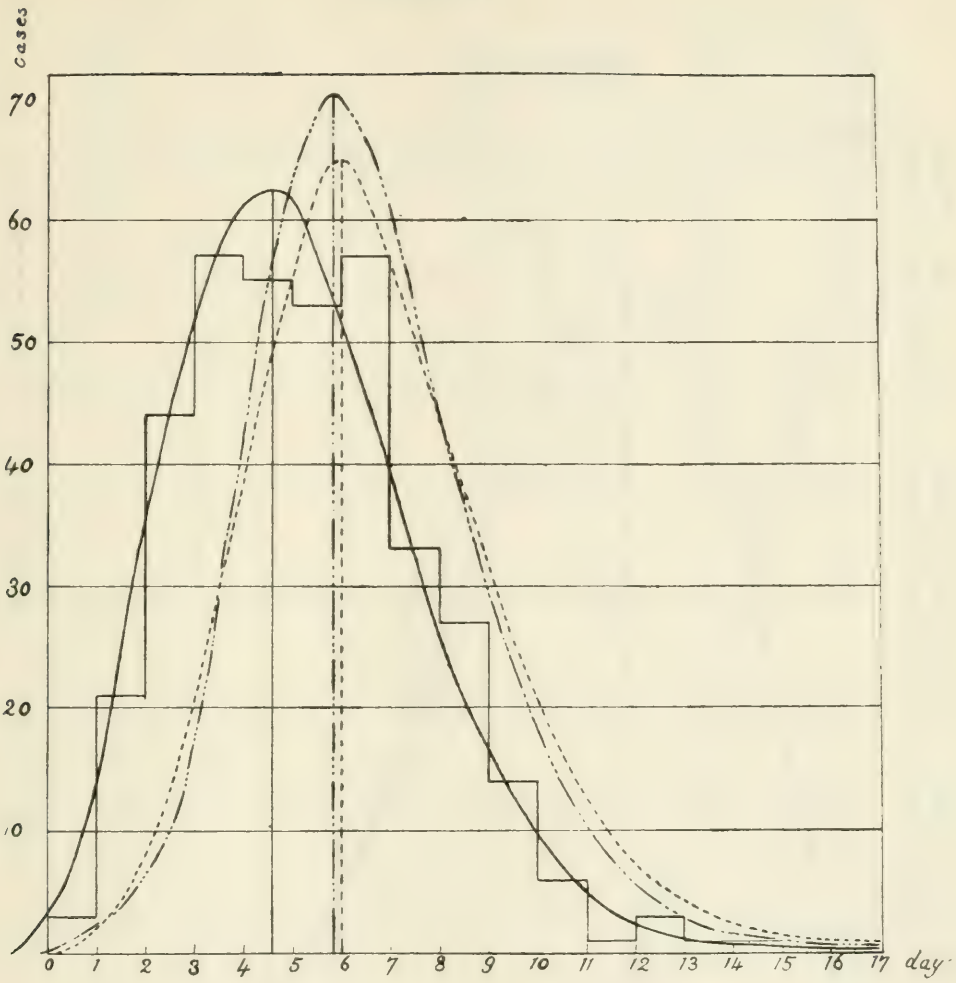
Death-rate per 1,000 for month. Average of five years. 1908-1912.

CHART VI



Percentage of total death-rate occurring in each month. Native figures based on five year average. U.S.A. and German distributions, January read as July, etc.

CHART VII.



Showing day on which crisis occurred in 378 cases of pneumonia amongst Natives from tropical areas, on the Rand. For comparison two curves for distribution of termination by crisis from European figures are given reduced in scale, so that total area of all three curves are the same.

———— = Native data.

—— - - - - - = Musser & Morris 4718 cases.

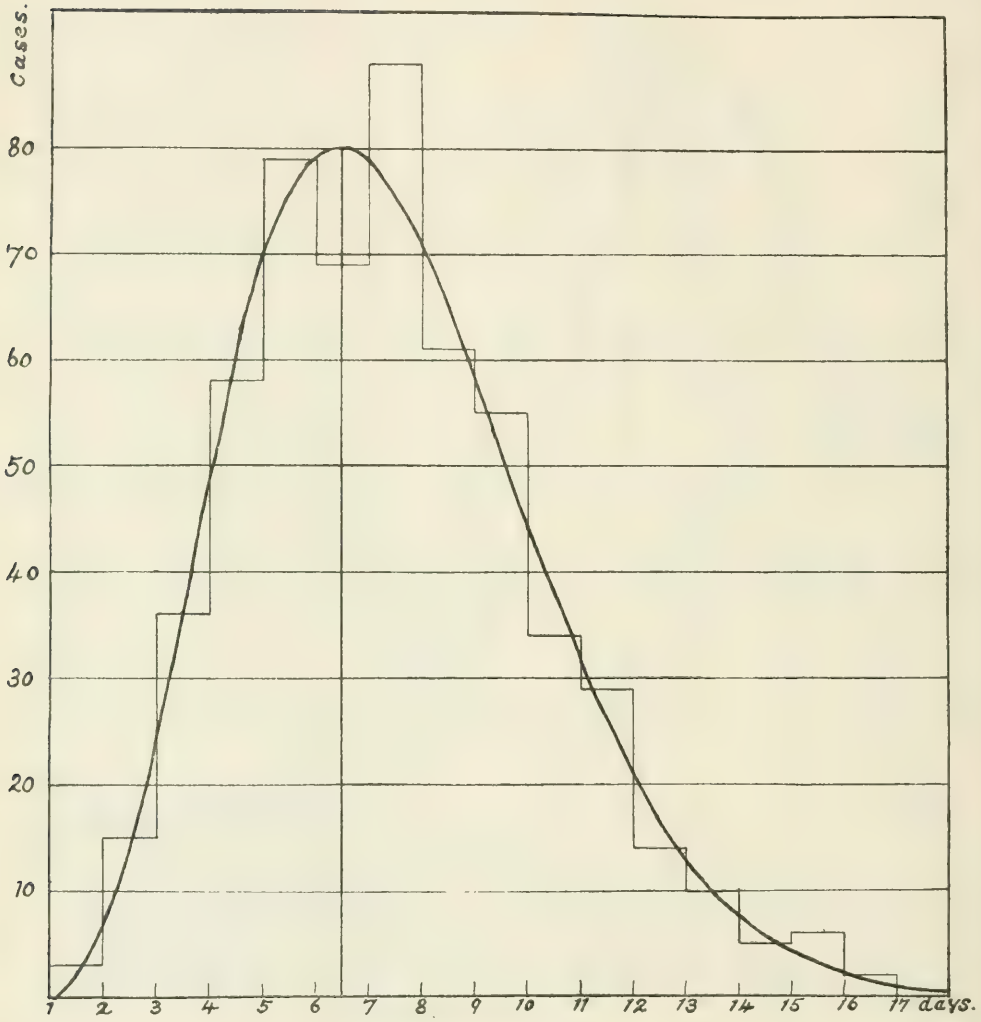
. . . . . = Aufrecht 873 cases.

Natives $y = 62{\cdot}26\left(1 + \frac{x}{5{\cdot}6590}\right)^{4{\cdot}2934}\left(1 - \frac{x}{25{\cdot}7944}\right)^{19{\cdot}5700}$

Musser & Morris $y = {\cdot}006108\left(1 + \frac{x^2}{40{\cdot}9510}\right)^{-10{\cdot}9291} e^{24{\cdot}5806 \tan \frac{x}{6{\cdot}3993}}$

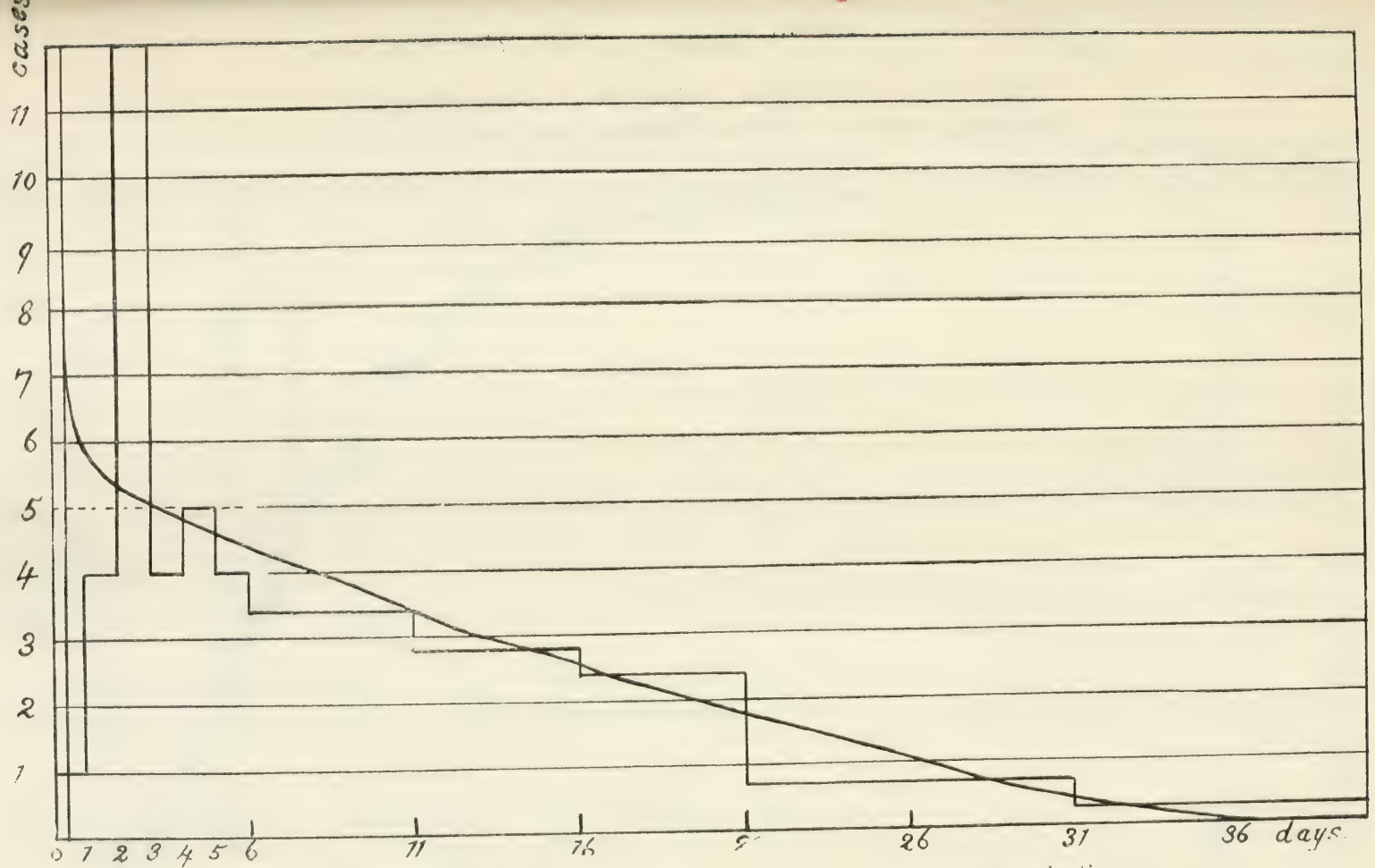
Aufrecht $y = 4{\cdot}4340\left(1 + \frac{x^2}{53{\cdot}3981}\right)^{-7{\cdot}8771} e^{10{\cdot}8782 \tan \frac{x}{7{\cdot}3074}}$

CHART VIII.



Showing day on which the temperature reached the normal by lysis, in 564 cases of pneumonia amongst "Tropical Natives" in W.N.L.A. Hospital.

$$y = 79{\cdot}84 \left(1 + \frac{x}{5{\cdot}8624}\right)^{3{\cdot}2072} \left(1 - \frac{x}{22{\cdot}7921}\right)^{12{\cdot}4691}$$



Second attacks of pneumonia according to number of days after termination of pyrexia of first attack. Cases in W.N.L.A. Compound Hospital.

$$y = 5{\cdot}912 \left(\frac{x}{1{\cdot}1691} - \right)^{-{\cdot}0391} \left(1 - \frac{x}{36{\cdot}4804} \right)^{1{\cdot}2201}$$

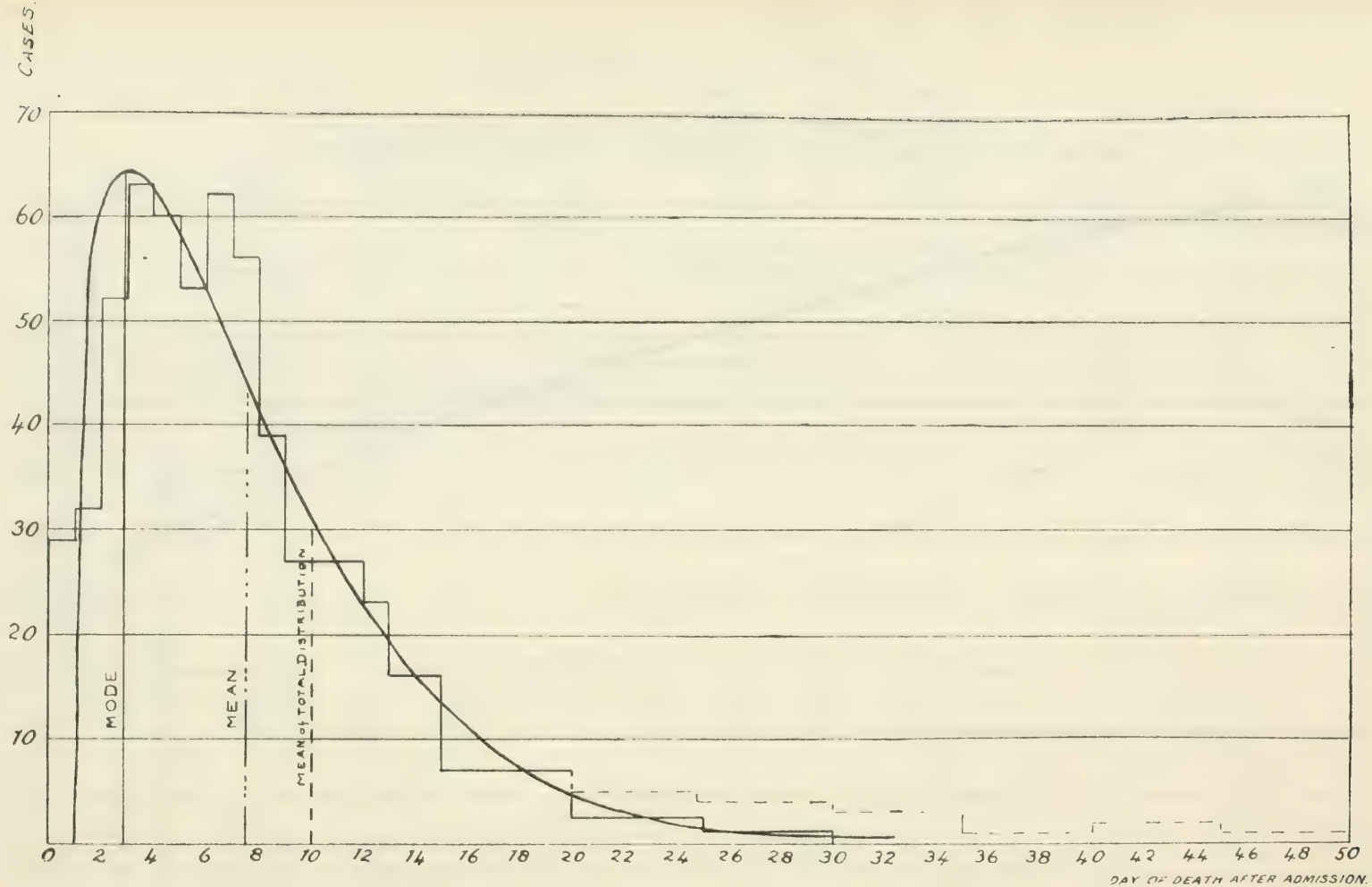


CHART X.

Day of death after admission to W.N.L.A. Hospital. Pneumonia.

All cases N=705. J_1 Start 3·0915. End 103·8423 Mean 10·0504.

Dotted tail omitted N=640. I. $y=64{\cdot}25\left(1+\frac{x}{2{\cdot}1761}\right)^{{\cdot}3568}\left(1-\frac{x}{54{\cdot}9565}\right)^{9{\cdot}0110}$

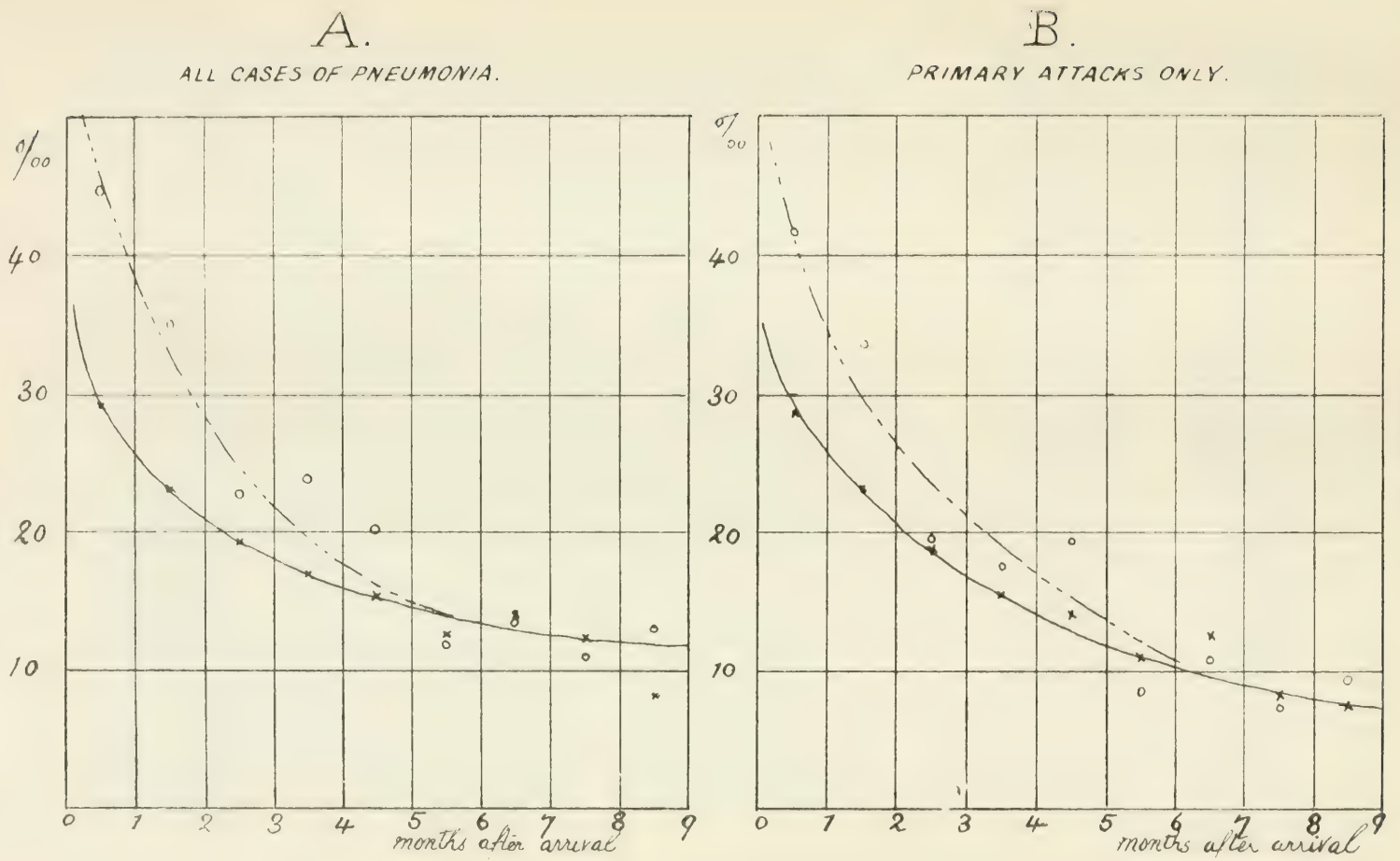


CHART XI.

Attack rates per 1,000, for months after inoculation on arrival at W N.L A Compound.

SPECIFIC SEROLOGICAL REACTIONS WITH PNEUMOCOCCI FROM DIFFERENT SOURCES.

Being a method for demonstrating the presence of specific opsonins and agglutinins in the sera of pneumonic patients: and a description of its application in differentiating the pneumococcus into distinct groups.

By F. S. LISTER, M.R.C.S., L.R.C.P.

During the latter part of 1911 and the early part of 1912 I was engaged as an assistant to Sir Almroth Wright, who was then carrying out researches into lobar pneumonia as it affected the native labourers on the Witwatersrand. Whilst so engaged one was struck with the comparative rarity with which pneumococci recently isolated from cases of lobar pneumonia were satisfactorily opsonised, even by the serum of pneumonic patients. On two occasions, however, I obtained opsonic preparations of a quite exceptional character, which showed massive agglutination of cocci, together with such a degree of phagocytosis that opsonic enumeration was impossible.

In June of the present year I set myself to prepare, if it were practicable, sensitised vaccines of the pneumococcus. For this purpose it was desirable to obtain a highly immune serum, or at least a serum having the properties which I had found on the two occasions referred to above. It occurred to me that a serum possessing these characteristics might be secured from a patient at, or about, the time of his crisis. The sera of five such patients all failed to give any reaction, such as described above, when tested with a stock culture of pneumococcus which had been recently isolated, by blood-culture, from a case of pneumonia. This culture was also tested with four normal sera with similarly negative results; it was, however, pathogenic to mice. Casting about for an explanation of this phenomenon, it occurred to me that I might obtain the reaction which I sought for if I were to make use of the patient's own pneumococcus and employ his own serum in carrying out the test. In this investigation I have obtained cultures of the pneumococcus by lung-puncture in twenty cases of lobar pneumonia, and I have also obtained samples of serum

from the same patients at different periods of their disease. The reactions of these various pneumococci and sera, one with the other, form the principal subject of this communication. It should be remarked that my twenty cases yielding the pneumococcus were not necessarily consecutive cases of pneumonia; for the performance of lung-puncture was not always productive of a growth of the pneumococcus, and such cases were, therefore, of no use for my purpose.

Technique. Wright's opsonic method(1) as carried out by means of the "teat and capillary glass tube" is the one which I have adopted in my enquiry. By one practised in the technique the testing of a large number of sera can be quickly performed; this is an important consideration, as the number of specimens to be examined increases rapidly with each fresh case investigated. An additional advantage of the method is that it enables both agglutination and phagocytosis to be observed in the same preparation.

In order to secure uniformity, and to exclude as far as possible the chance of a reaction occurring with normal sera, the following conditions were adhered to throughout:—

(1) The pneumococci were grown on human-blood-agar. I have noticed that such cultures are less susceptible to phagocytosis than those grown on serum or ascitic-agar.

(2) The emulsions of cocci were made in 0·85 per cent. saline, and were so prepared as to be free from "clumps" and "chains." If distilled water be employed a much greater degree of phagocytosis is liable to occur, especially with certain strains of the organism.(2)

(3) The phagocytic mixtures were incubated for fifteen minutes.

(4) The phagocytic mixtures consisted of equal volumes of blood corpuscles, emulsion of pneumococci, and unheated serum.

(5) The blood corpuscles were obtained in every case by pricking the skin of my own finger.

(6) The blood was collected into 1·5 per cent. sodium citrate solution, the mixture was centrifuged and the deposited corpuscles were washed twice with 0·85 per cent. saline.

(7) The sera from patients and the "control" were collected by means of Wright's blood-capsules after puncturing the skin of the finger.

(8) The source of "control" serum was always my own blood.

(9) The cultures of pneumococci made use of have been obtained by lung-puncture only.* That the cultures should be obtained either

* Without expressing any opinion of the safety of this procedure as a routine method of obtaining a bacterial sample, I may state that in only one instance out of a total of thirty-three was the operation followed by any untoward result. In this case a somewhat alarming hæmoptysis supervened, and the respiration became embarrassed. These symptoms, however, subsided within six hours, and the patient made an uneventful recovery from his pneumonia.

by lung-puncture or blood cultivation is a condition which I consider to be of considerable importance, for a culture which is obtained from the sputum may possibly represent no more than the pneumococcus which is frequently present in the throats of healthy persons; the latter event is certainly possible if the sputum has not been thoroughly washed. Pneumococci from the nose and pharynx are presumably less virulent than those obtained from pulmonary lesions.

It might be thought permissible to make use of cultures obtained by inoculating mice with the pneumonic sputum, but the fact must be borne in mind that the pneumococci from the noses and throats of even healthy people occasionally prove fatal to these animals.

Throughout the experiments which I shall now briefly record each case of pneumonia was given a serial number, and the same serial number was also used to designate both the serum and the pneumococcal growth yielded by the same patient.

Case I. "Tropical" Native. Lobar Pneumonia. Pneumococci isolated by lung-puncture (Culture I.). Serum (Serum I.) collected immediately after crisis.

The serum of the patient and also the "control" (normal) serum were tested with Culture I.—that is, the patient's own organism. With the "control" serum there was practically no phagocytosis (0/50), neither was there any agglutination. With the patient's serum, phagocytosis was so excessive as to be uncountable, whilst agglutination was very marked. The agglutination took the form of areas of compacted cocci, and some of these areas were so large that they covered the entire microscopic field.

Case II. Pondo Native. Lobar Pneumonia. Pneumococci isolated by lung-puncture four days before crisis (Culture II.). Serum collected and tested daily.

The serum on the four days preceding the crisis produced no obvious reaction with the organism, but on the day on which the temperature began to fall massive agglutination together with uncountable phagocytosis occurred. The temperature on the following morning was normal, and the patient presented all those symptoms of improvement which are usually associated with recovery by crisis. The "control" serum did not exhibit at any time the slightest degree of opsonic or agglutinative action.

Although I have stated that the patient's serum presented no obvious reaction on the four days preceding the crisis, yet a sample which was collected twelve hours before the fall in the temperature commenced showed some signs of agglutination, there being many clumps of five to eight cocci distributed along the edge of the opsonic film. A similar phenomenon did not occur in the "control" preparation.

The critical serum of Case I. when tested with Culture II., and the critical serum of Case II. when tested with Culture I., gave no evidence of reaction.

Cases III. and IV. Pondo and Baca Natives respectively. The pneumococcus was obtained from both of these some days before the crisis. The behaviour of these organisms, as to their serological reactions, was similar to that described under Case II. The serum from Case I. again failed to produce any reaction with either Cultures III. or IV., and Sera III. and IV. failed to react with Culture I., despite the fact that they had developed the power to act with Cultures III. and IV. I found, however, that any of the Sera II., III., or IV., reacted typically with any of the Cultures II., III., or IV. Such a result suggested that one had to deal with groups or strains of pneumococci. It appeared that these strains of pneumococci differed from one another in their reactions with immune sera, a difference which

was both striking and easily recognisable under the microscope. The application of such a method to the identification of different groups of pneumococci would have very definite advantages, as the criterion would be the presence or absence of very marked phenomena, and not the estimation of slight gradations or variations in a picture common to all the observations. The error which inevitably arises from the personal equation is therefore avoided.

After my experiments with the four cases as described above, my objective became definitely settled in the direction of the search for: (1) The existence of groups of pneumococci having distinct serological reactions; (2) the presence in " critical " sera* from pneumonic patients of specific opsonins; and (3) the presence of specific agglutinins in " critical " sera from pneumonic patients.

Upon a perusal of the accessible literature a number of references bearing upon these subjects have been found. Thus I note that:—

J. H. Musser and G. W. Norris(3), referring to Rosenow, state that he was unable to find phagocytosis of virulent pneumococci by normal or by the patient's serum until the organism had been attenuated by cultivation. He states that pneumococci which had been isolated from the patient's blood, before, during, or after crisis, are not *taken up* (*sic*) either by normal or the patient's serum *in vitro*.

W. H. Park and Anna W. Williams(4) have suggested the possibility of separating pneumococci into groups by means of differences in their morphological characteristics; they also place Streptococcus mucosus capsulatus amongst the pneumococci; and referring to agglutination and quoting Collins, conclude that the phenomenon is so variable as to be of no practical valu .

L. Cotoni and C. Truche(5) found that the agglutinative power of different sera for different strains of pneumococci varies greatly, and that there is no regularity in the results obtained: that the agglutination method is therefore unsuitable for the diagnosis of pneumococcal infections in man. They found agglutinative reactions in 25 per cent. of sera from cases of human lobar pneumonia. Certain paradoxes occurred; for instance, some sera agglutinated a strain of pneumococcus when the serum of the patient from whom the organism was obtained failed to do so. The results obtained with normal and immune horse serum were similarly paradoxical.

Rufus Cole,(6) reviewing the method of recovery in lobar pneumonia, discusses the cause of the crisis; he finds that attempts to demonstrate an increase of the ordinary bactericidal substances, which act in conjunction with complement, have failed. He points out that univalent immune serum protects only 40 per cent. of animals in

* It will be seen from the charts that opsonins and agglutinins are also present in the sera of patients recovering by lysis; the designation " critical " as applied to sera is a mere verbal convenience, and should not be taken literally.

experimental inoculations; he infers from this that at least 60 per cent. of cases are due to organisms other than those of the type strain.

A. B. Wadsworth(7) in an analysis of some agglutination experiments with pneumococci, and dealing with the sera of forty-six cases of pneumonia and fourteen "controls," found that ten out of the fourteen "controls" agglutinated the pneumococcus. From such a result he concluded that the agglutination test in pneumonia is of very little practical value. The same author satisfied himself experimentally that virulent pneumococci are practically insusceptible to phagocytosis. He quotes Hektoen's opinion that insusceptibility to phagocytosis is an indication of virulence and that the opsonic index may therefore be the measure of the virulence. Wadsworth, however, found that virulence may be lowered without any increase in susceptibility to phagocytosis.

S. Strouse(8) refers to Neufeld's work, by which the latter claims to have demonstrated the presence of "immune opsonins," or bacteriotropins, in almost every sample of pneumonic serum. In some later work, however, strains of pneumococci were found which were not acted upon by sera which ordinarily gave a positive result with other strains; such pneumococci were, nevertheless, acted upon by the post-critical sera of the pneumonic patients from whom they had been derived.

Seligmann and Klopstock(9) and Boettcher(10) were unable to confirm these results. Strouse, following Neufeld's technique, was never able to find opsonins in heated sera which had been taken after the crisis, and tested with virulent pneumococci which showed no spontaneous phagocytosis. From further animal experiments he was able to show that phagocytic immunity is specific, to a high degree, for the organism used in immunisation, and that the amount of opsonin produced largely depends on the virulence of the organism.

A. R. Dochez(11) found that the serum of pneumonic patients at their crisis protected mice, and that the presence of protective substances is more evident when the homologous organism is used for the test. In only four cases—out of, apparently, fourteen cases examined—were protective substances demonstrated before the crisis. Quoting Neufeld, he says that pneumococci have not as yet been differentiated into clearly defined biological groups, though it is known that a serum derived from a certain typical strain may fail to protect animals against an infection by other strains which are morphologically and culturally characteristic.

Bezançon and Griffin(12) state: "Agglutination is not obtained with all strains of the pneumococcus; it is, in a way, an individual and

not a specific property; very often agglutination is obtained with the micro-organism recovered direct from the infected person while laboratory strains give negative results."

The conclusions which I draw from such references are—(1) That agglutination, although of frequent occurrence, is too variable a phenomenon to be of practical use. (2) That virulent pneumococci are not susceptible to phagocytosis in any circumstances. (3) That immunity, as studied by animal experiments, is specific, in great measure, for the particular strain of pneumococcus with which the animal has been inoculated.

Having dealt with the experiments and conclusions of other workers, I have now to refer to my own observations. Both the agglutinins and the opsonins in the serum of pneumonic patients appear to be specific for certain classes of the pneumococcus. The serum of patients suffering from lobar pneumonia seems to possess marked agglutinative and opsonic properties with respect to the particular organism responsible for the infection: provided always that the serum is collected about the time that the temperature falls, and provided also that this fall of temperature is not an indication of approaching death. There appear to be occasional exceptions to the rule, but the probable infrequency of such exceptions can be seen by reference to the tabulated statement of my experiments.

In addition to this specific reaction of a critical serum with its particular organism, a cross reaction can be obtained with other organisms and other sera. Thus a critical serum when tested with a number of strains of pneumococci, isolated from other cases of pneumonia, will affect some of these pneumococci in a similar manner, whilst with others there will be no trace of reaction. If now the sera of the patients from whom the reacting organisms were obtained, be tested with the particular organism which was clinically associated with the critical serum first mentioned—provided that these sera be collected at a time when they react to their own particular organism—a similarly positive result will be obtained. To put the matter in another way. If a, b, c, and d are four cultures derived from four different cases of lobar pneumonia, and if a′, b′, c′, and d′ represent the sera of these patients—and granting for argument's sake that the serum of each of these patients has developed an agglutinative and opsonic reaction towards his own organism—then, if the serum a′ show a reaction with organisms b and c, but not with d, it will also be found that the sera b′ and c′ react with organism a. Sera a′, b′, and c′ will not react with organism d, nor will serum d′ react with any of the organisms a, b, or c. In other words, the reciprocal action of organism and serum is specific.

I applied such a method of serological investigation to the twenty strains of pneumococcus to which I have already referred; the technique was as I have previously described. By such means I found it possible to classify eighteen of the strains into four definite groups. Two of the strains were, however, quite refractory and gave no indication of being affected either by the serum of the patients from which they had been derived, nor by any of the sera in my possession. Both of the cases which yielded these refractory strains came from the same compound; both recovered, one after a particularly mild attack, and the other after an illness of exceptional severity—in fact it seemed to only just escape a fatal issue. Both patients were members of a tribe whose susceptibility to and mortality from pneumonia, at any rate on the Rand, is very high. I have no explanation to account for the irregularity of my results with these two cases.

Among the twenty cases referred to there were four, from whom cultures were obtained, which terminated fatally without any evidence of crisis; the sera of these patients did not at any time exhibit any agglutinative or opsonic properties.

Two of my series of twenty died after exhibiting a crisis—one three weeks after, of tuberculosis of both lungs, the other two days after, with renewed pyrexia. The sera of both these cases exhibited the usual marked reaction shortly before the crisis and until death.

It will be seen, therefore, that there were sixteen cases, including the two exceptional cases referred to above, which terminated by either crisis or lysis, and that the sera of fourteen of these developed agglutinins and opsonins.

In carrying out the investigation a culture was tested as soon as it had been definitely isolated, an event which, in most cases, occurred several days before the serum from the case had devloped any activity. The cultures were also tested with sera derived from other cases, and which were known to have developed activity towards their own organisms. In every case so tested, where a positive reaction was obtained, the serum of the case of origin subsequently developed agglutinins and opsonins, not only for its own organism, but also for the organisms from all cases whose sera affected it.

From a consideration of these phenomena it seems reasonable to assume that the sera of the four cases which terminated fatally, and whose particular organism gave a reaction with one or other of the potent sera, would have developed their own agglutinins and opsonins had the patients survived. It may be that these phenomena are very closely associated with recovery, although one cannot, at the present, define the changes of which they are the indicators.

In the earlier cases an attempt was made to estimate the degree of phagocytosis, in both the sample and "control" preparations, by ascertaining the bacterial content of 50 leucocytes on each slide. It was found that the preparation made with normal serum usually contained not more than one phagocyted coccus in fifty cells, and that very rarely was this number raised to ten. In preparations made from the patient's serum, however—when such had become active—the intra-cellular collection of cocci were, in all cases, so numerous as to render accurate estimation impossible. Although empty cells were not uncommon, yet cells each of which contained from ten to forty cocci were numerous.

As it appeared of no practical value, for the purpose of this experiment, to attempt an opsonic estimation, and as, moreover, the probable error would have been enormous, systematic enumeration was not attempted. It may be stated, in general terms, that fifty leucocytes with "control" serum contained from nought to ten cocci, whilst with the patient's serum cocci to the number of two hundred and fifty and upwards would have to be enumerated.

It has been urged that agglutination occurring in a phagocytic mixture leads to misinterpretation when the film comes to be enumerated, for in such circumstances the appearance of from ten to twenty cocci in a single cell may be due to masses of agglutinated cocci lying either above or below the leucocyte. I am satisfied that this contention is, to a large extent, unwarrantable, and for the following reason. During my investigations agglutinins have often been demonstrated to be present before opsonins have appeared, and they have persisted after the latter have disappeared. Opsonic films prepared in such circumstances have not shown any indications of apparent phagocytosis.

It seems probable that agglutination may in part account for the irregularity of the phagocytosis in films in which many empty cells are seen. Its action in this matter may be purely physical and due to the fact that by causing the collection of the cocci into large clusters it withdraws them entirely from the field of action of some of the leucocytes.

In my observations I have found no difficulty in deciding on the presence or absence of agglutination, for whenever it was present the appearances were unmistakable. Some sera which were obtained when agglutinins were only just appearing provided films containing numerous groups of from five to eight cocci scattered throughout the field. It was the rule, however, for further samples of such sera, taken within twenty-four hours of the first and sometimes earlier, to possess the power of causing massive agglutination.

The term *Virulent*, as applied to pneumococci, appears to be used in current literature with variable and uncertain meaning. The majority of authors appear to refer the quality of virulence of the pneumococcus to its capacity to kill the mouse, while others seem to consider any pneumococcus virulent which can or has produced pneumonic changes. I leave this terminological dispute undecided and merely use the word as an epithet to organisms of the latter class. It is doubtful whether virulence for mice and capacity for producing lobar pneumonia are parallel characteristics, and it is also doubtful—at all events to me—whether a virulent pneumococcus is ever phagocyted or agglutinated, *in vitro*, by a normal serum.

With regard to the twenty cultures of the pneumococcus which I have studied, and which were all derived by lung-puncture from cases of pneumonia, the following facts may be recorded. None of them were susceptible to phagocytosis, either by the control serum used, or by any of the ten other normal sera which will be subsequently referred to. Nine were inoculated subcutaneously into mice, and all proved fatal in from eighteen to sixty hours. One culture, originally insusceptible to phagocytosis by normal serum, subsequently developed this property in a marked degree. Before this change came about it had been subjected, however, to many subcultivations on human-blood agar, over a period of two months. After it had become opsonisable by normal serum it was found to be still virulent for mice, although a larger dose was now necessary, and the period of survival was prolonged.

For the sake of brevity in describing the following experiments the expression " the reaction " is used as referring to the presence of obvious agglutination and phagocytosis. An opsonic film was made use of to demonstrate the presence or absence of the reaction, and the conditions of the preparation of the phagocytic mixture have already been described ; in two instances, however, the sera, after being tested in the usual manner, were diluted forty-fold, with the result that phagocytosis was completely inhibited, although some agglutination still remained apparent. No systematic study was, however, made of the effect on the character of the reaction of variations in the degree of dilution of the sera.

The following table shows the presence or otherwise of the reaction in the sera of fourteen pneumonic patients, *all of whom recovered*. In every case the serum was critical or post-critical, and the organism with which it was tested was always derived from the patient who yielded the serum. The " control " observation on each occasion was made with normal serum and the organism of the case.

TABLE I.

Case No.	Tribe.	"Reaction."	"Control reaction."
1	Mozambique	+	—
2	Pondo	+	—
3	Baca	+	—
5	Pondo	+	—
7	M'Sutu	+	—
8	Quilimane	+	—
9	,,	—	—
10	M'Sutu	+	—
11	Zambesi	+	—
12	M'Sutu	+	—
13	Zulu	+	—
17	Nyambaan	—	—
19	Pondo	+	—
20	Zulu	+	—

The results of testing the sera of six cases, *all of which terminated fatally*, are given in Table II. The conditions of the test were the same as those recorded in Table I.

TABLE II.

Case No.	Tribe.	"Reaction."	"Control reaction."
4	Pondo	+	—
6	X'osa	—	—
14	X'osa	+	—
15	M'Chopie	—	—
16	M'Chopie	—	—
18	Mozambique	—	—

Of the two cases which yielded the reaction, patient No. 4 died three weeks after the crisis of acute pulmonary phthisis, and patient No. 14 died two days after crisis with a fresh elevation of temperature. None of the other four cases afforded the reaction, and none of them had reached the stage of defervescence.

Table III. records the results, with respect to the presence or absence of reaction, of testing a series of pneumonic sera with a series of pneumococci derived from the same cases.

TABLE III.

No. of case providing test serum.	Case number of origin of culture.									
	1	2	3	4	5	7	8	11	12	Control.
1	+	—	—	—	—	—	—	+	—	—
2	—	+	+	+	+	+	—	—	—	—
3	—	+	+	+	+	+	—	—	—	—
4	—	+	+	+	+	+	—	—	—	—
5	—	+	+	+	+	+	—	—	—	—
7	—	+	+	+	+	+	—	—	—	—
8	—	—	—	—	—	—	+	—	—	—
11	+	—	—	—	—	—	—	+	—	—
12	—	—	—	—	—	—	—	—	+	—

It will be seen from this table that all the organisms tested arrange themselves into four groups according to their reaction with the sera. These groups, which will, hereafter, be designated A., B., C. and D., comprise the organisms from the following cases. Group A., from cases 2, 3, 4, 5, and 7; Group B., from cases 1 and 11; Group C., from case 12; and Group D., from case 8.

In subsequent work I have been content to test any new culture of the pneumococcus by its reaction with four sera, each of which was representative of one of these four groups. Though many experiments were done, from time to time, on the extended basis shown in Table III., yet no serum was ever found which transgressed the boundaries of the group-specifity which I have found to exist.

The cultures from cases 9 and 17 were tested with every available active serum and also with the sera obtained from twelve apparently normal Europeans and Natives. None of the sera produced any reaction with these organisms, and, as already stated, no demonstrable agglutinins and opsonins appeared in the sera of the cases from which they had been derived. For these reasons it was impossible to classify them in any of the first four groups; a fifth group, which I have called "E," was therefore set aside for the reception of such anomalous strains.

Out of the twenty cultures tested by the above method, eighteen were found to be classifiable into one of four groups, as follows:—

A. 2. 3. 4. 5. 7. 10. 13. 14. 19.
B. 1. 11. 16. 18. 20.
C. 12.
D. 6. 8. 15.

The numbers which designate the cultures in the above statement represent also the serial order in which the cases from which they were derived came under my observation. It will be seen that a repre-

sentative of each group was discovered in the first twelve cases examined, and that in the eight subsequent cases no new group was discovered. This fact suggests that amongst the population dealt with, and at the present time, the number of groups is limited.

The cases were drawn from Native mine labourers employed on various mines on the Rand, and from Native "house and outside boys" working in Johannesburg.

When these groups had been established a representative culture from each was tested with each of the sera from five healthy Natives and five healthy Europeans, and the reaction was found to be absent on all occasions.

In the application of this method of investigation it is of great advantage to have access to a large number of pneumonia patients within a short period, for the opsonins and agglutinins seem to disappear from the freshly drawn serum at the end of a few weeks. With respect to the retention of these substances by serum *in vitro*, I have a sample which was collected at the time of the crisis and which is now ten weeks old. No special care has been taken of this sample as it has been lying on the bench. I find that the opsonic power has now disappeared, but that sufficient of its agglutinating powers remain to enable me to use the sample in detecting any organism belonging to Class B. Serum from blood which has been collected in the ordinary manner, in a Wright's capsule, and allowed to stand and clot, is still quite active after several days. I have found that some cultures, after many transplantations, become susceptible to phagocytosis by normal sera, although I have not found any cultures which developed liability to agglutination; the phenomenon of agglutination is, therefore, of greater service than that of phagocytosis for the purpose of classifying organisms.

The opsonic power of serum is, with few exceptions, destroyed by heating at 55° C. for twenty minutes. The agglutinating power, however, survives this treatment, although its activity appears to be somewhat reduced.

If the validity of this method of classifying pneumococci be confirmed it will have more than one useful application. The possibility of identifying the particular strains of the organism will provide a clue as to the source of infection in different cases, and will help to elucidate the rôle of those pneumococcal organisms which are found so commonly in the throats of healthy individuals. The prevalence of different groups of pneumococci in different circumstances—industrial, tribal, geographical, etc.—will be capable of investigation. The suggestion that second attacks of pneumonia are due to invasion by another strain of the organism can be put to the test. These and many similar enquiries may be rendered feasible.

The prophylactic pneumococcal vaccine now so extensively used on these fields may, in the light of these studies, become modified; it is obvious that the efficacy of such vaccine would be enhanced if use were made of the particular strain of organism to infection by which the labourers will be exposed. It may also be found advantageous to use certain definite combinations of strains. Again, the efficiency of sensitised vaccines will probably be secured by employing an organism belonging to the same group as that which has infected the patient.

The serum therapy of pneumonia, which has not hitherto afforded notable results, may derive additional importance when it can be used in so specific a manner as the results of these investigations lead one to hope.

CONCLUSIONS.

The sera of patients recovering from lobar pneumonia usually acquire opsonising and agglutinating properties for the particular strain of pneumococcus which is responsible for the infection.

"Virulent" pneumococci—by which I mean pneumococci which have been derived from a hepatized lung—are readily phagocyted and agglutinated in such circumstances.

Most pneumococci can be classified into groups by means of these specific serological reactions.

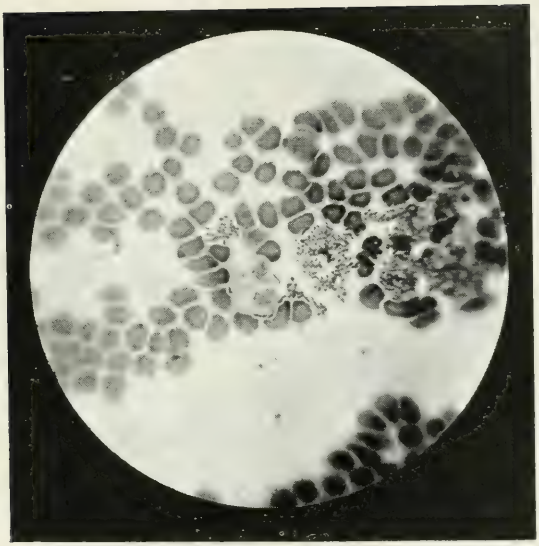
Certain strains of the pneumococcus cannot, at present, be classified by such means as the serum of the patients from whom they are obtained develops no opsonising or agglutinating powers.

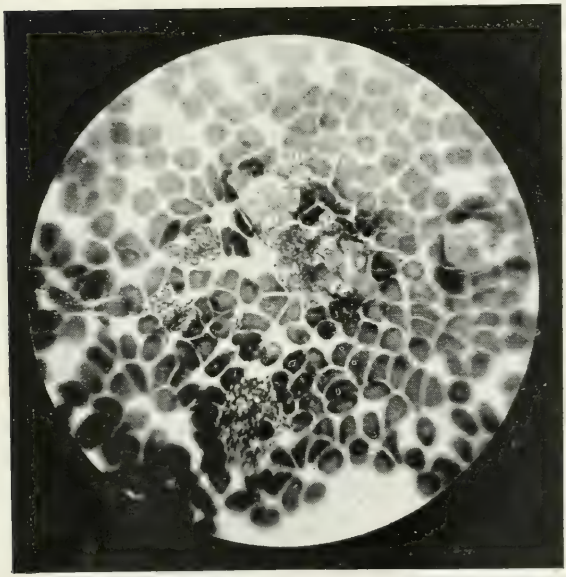
My work in carrying out this brief study has been much facilitated by the co-operation of Dr. W. Watkins-Pitchford, the Director, and it could not have been so advantageously conducted but for the generosity of the management of the Bantjes and Durban Deep Gold Mining Company in permitting me the necessary leave. Many of the cases investigated were kindly placed at my disposal by medical colleagues in the General Hospital and on the mines, and for this I am indebted to them. The photomicrographs are the work of Mr. O. Slawkowsky.

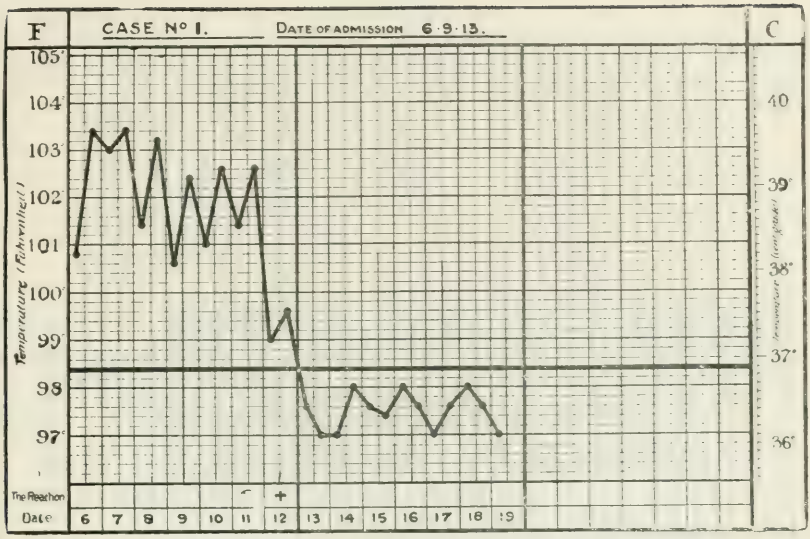
Postscript. Since committing the above study to paper my attention has been drawn to some recent work by A. R. Dochez and L. J. Gillespie, and which has appeared in the Journal of the American Medical Association, for September 6th, 1913 (pages 727 to 730). Although these workers have adopted different methods of investigation, yet their observations seem to have led them to conclusions somewhat similar to my own.

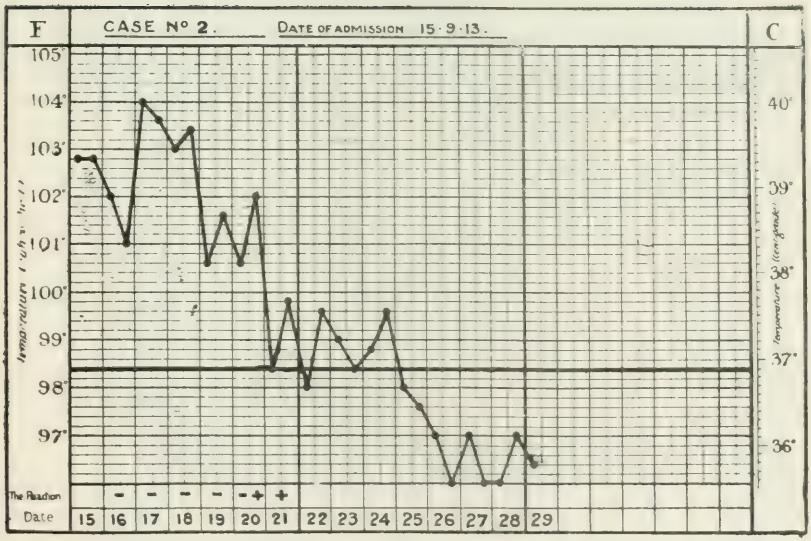
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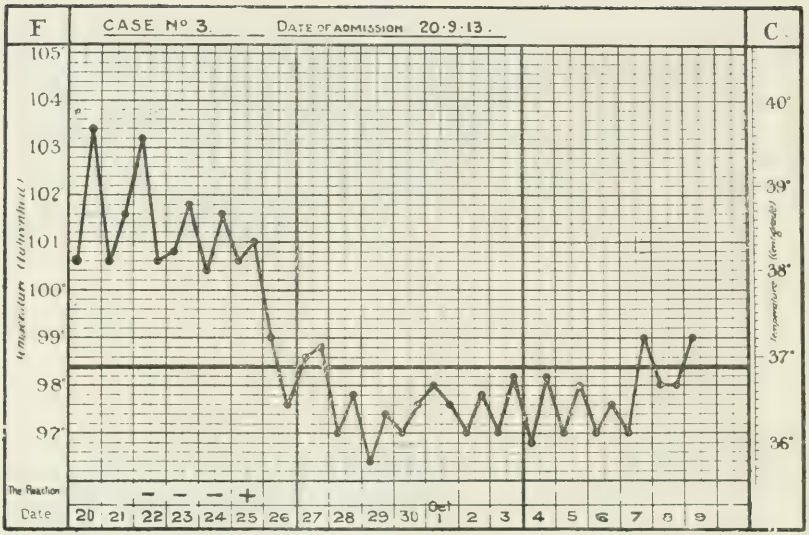
1. Sir A. E. Wright. *Technique of the Teat and Capillary Glass Tube* (Constable, London).
2. Observations by W. Parry Morgan in Sir A. E. Wright's *Report (Part II.) to Witwatersrand Native Labour Association*, Dec., 1913.
3. Osler & McCrae's *System of Medicine.*
4. *Pathogenic Micro-organisms*, 1910 Edition.
5. *Etudes sur le Pneumocoque, agglutination des Pneumocoques humains et animaux.* Ann. de l'Inst. Pasteur, Vol. 26, p. 313, 1912.
6. *Journal of American Medical Association*, August 31st, 1912.
7. *Journal of Experimental Medicine*, July 1st, 1912.
8. *Journal of Experimental Medicine*, August 1st, 1911.
9. *Ztschr. f. Immunitäts.*, 1909, IV., 103.
10. *Deutsch. Arch. f. Klin. Med.*, 1910, XCVIII., 93.
11. *Journal of Experimental Medicine*, November 1st, 1912.
12. Quoted in *Practical Bacteriology, Microbiology and Serum Therapy* (Besson).

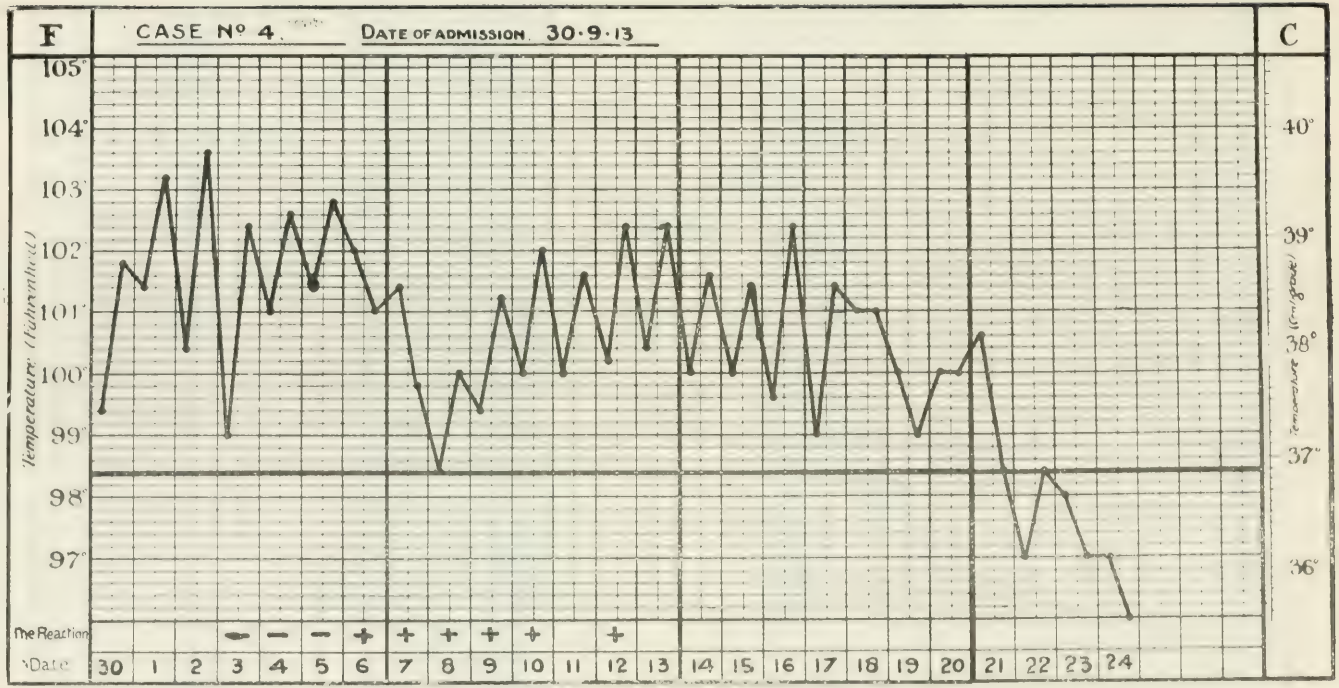


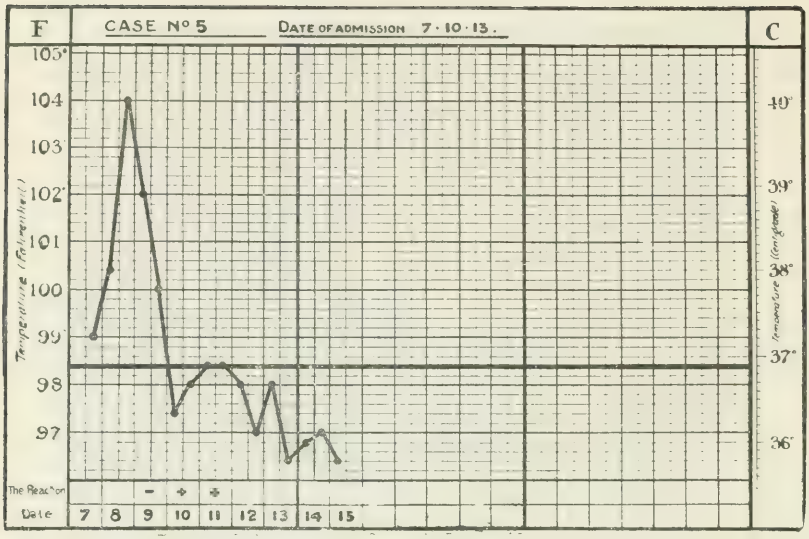


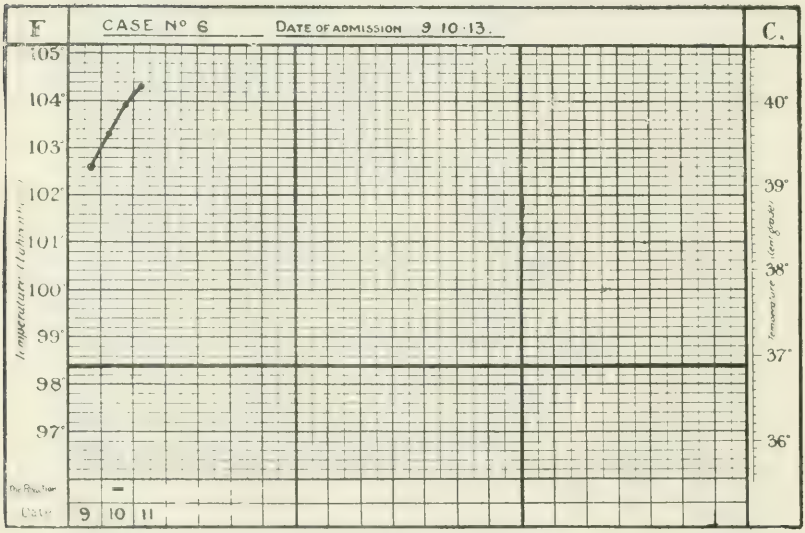

F
CASE Nº 1.
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Temperature (Centigrade)
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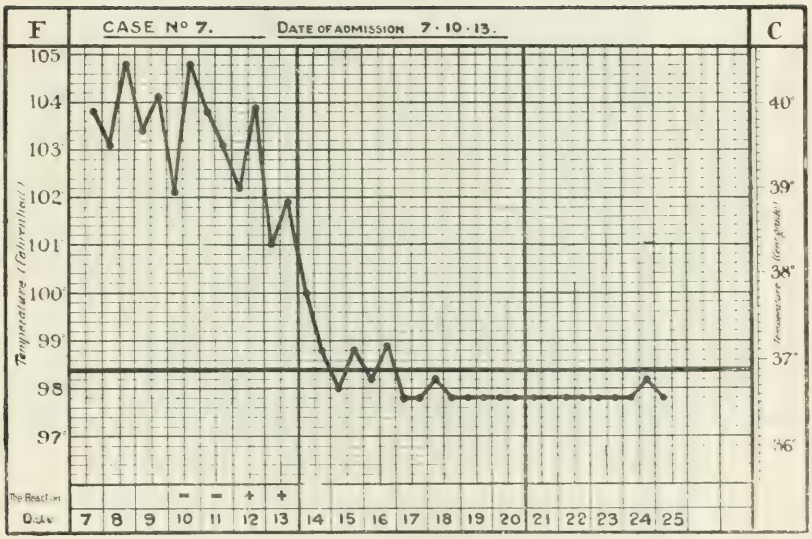

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Temperature (Centigrade)
The Reaction
Date
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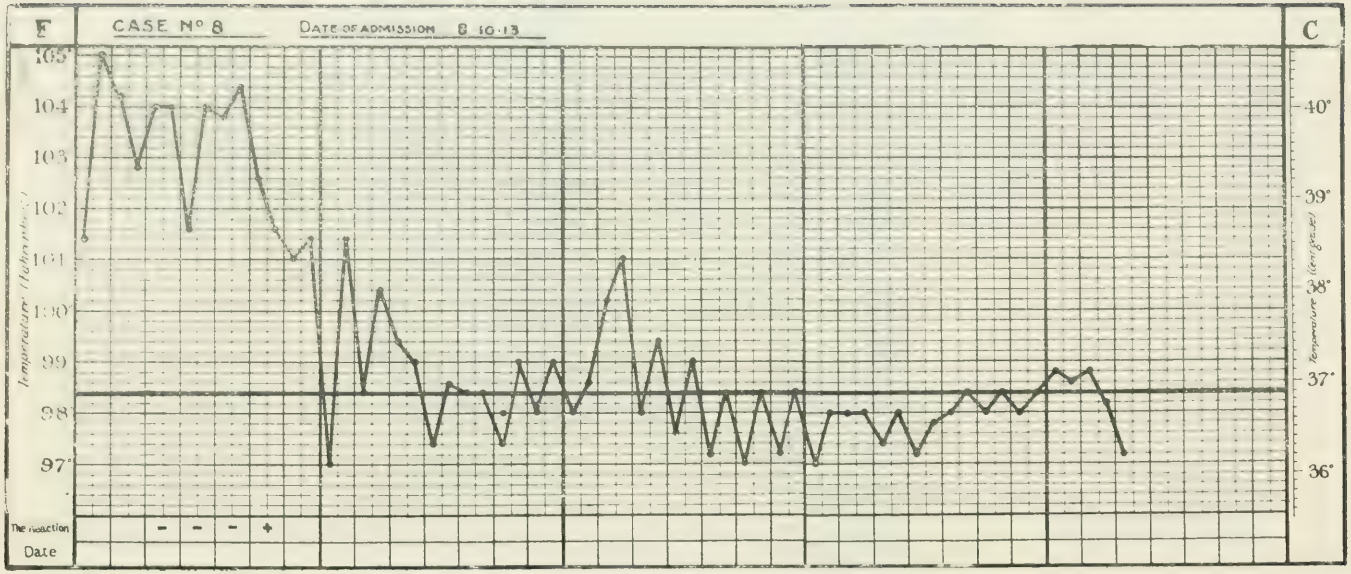

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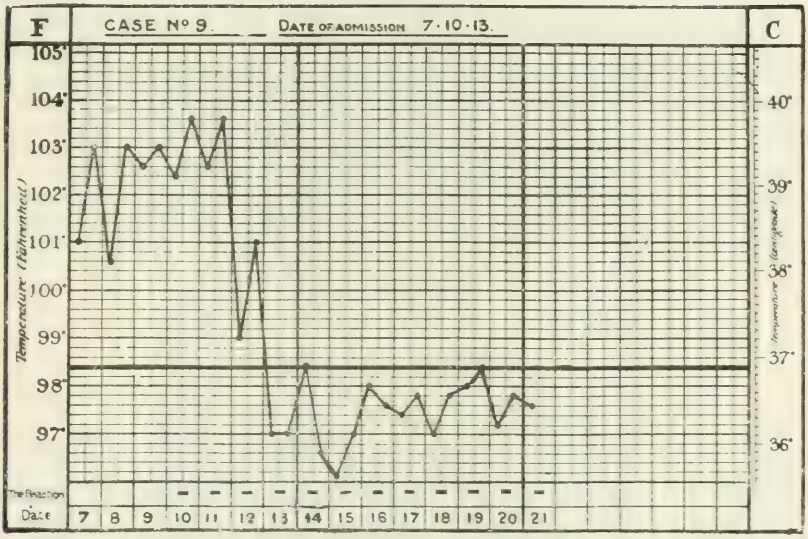

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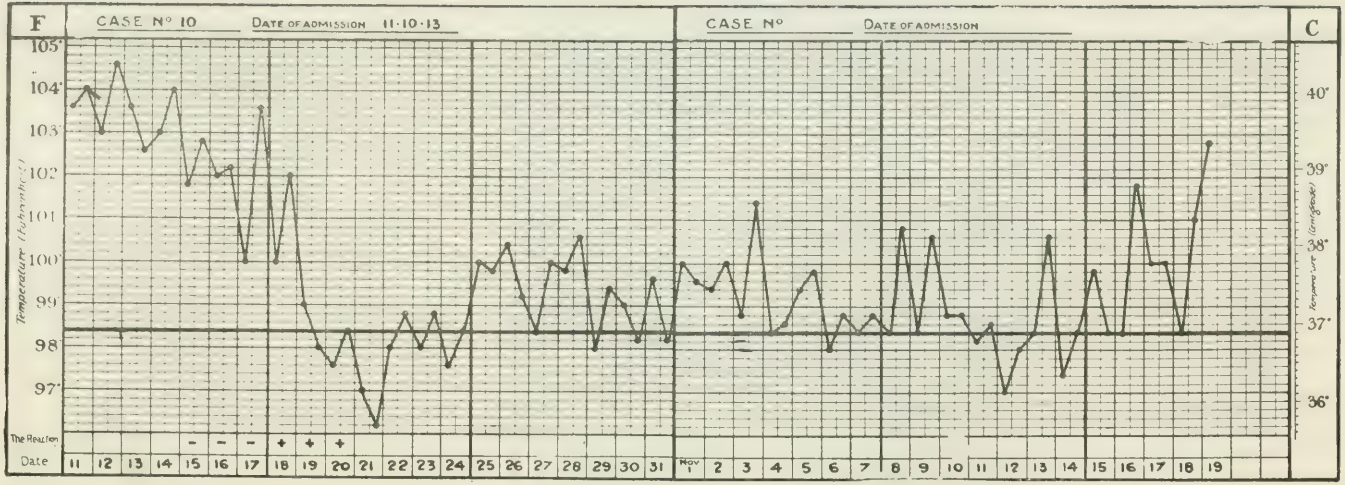

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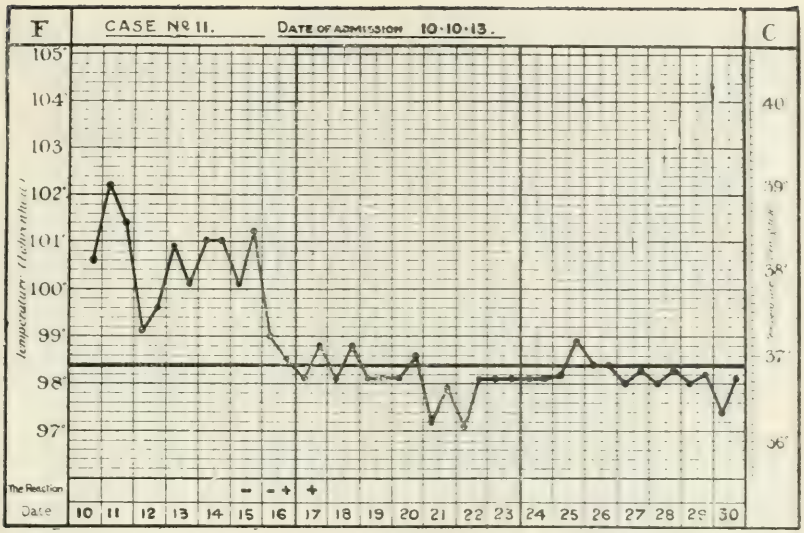

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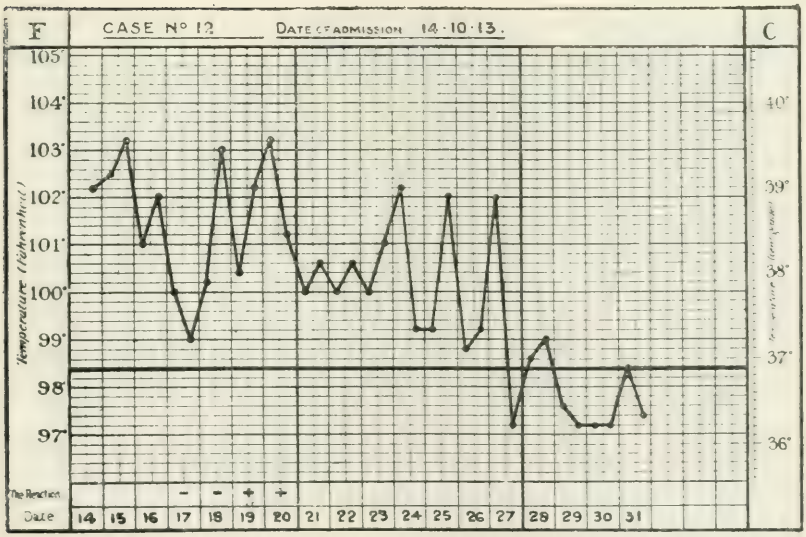

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Temperature (Fahrenheit)
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The Reaction
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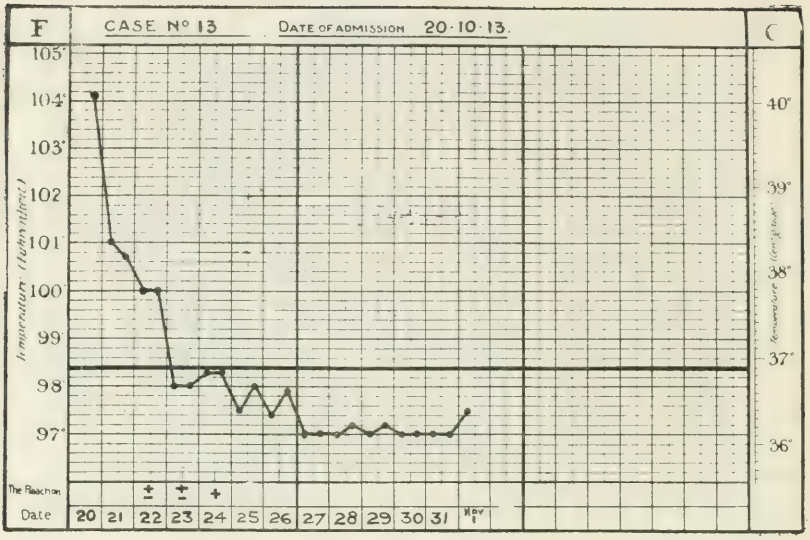

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DATE OF ADMISSION 8.10.13
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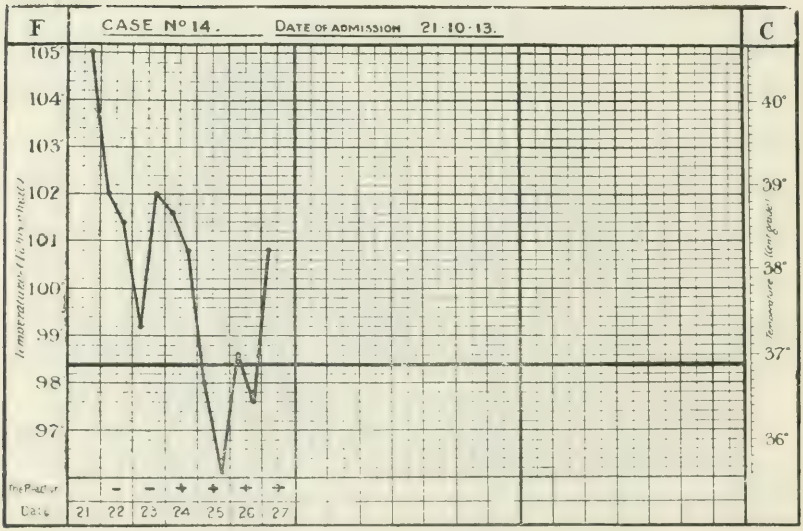

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Temperature (Centigrade)
The Reaction
Date
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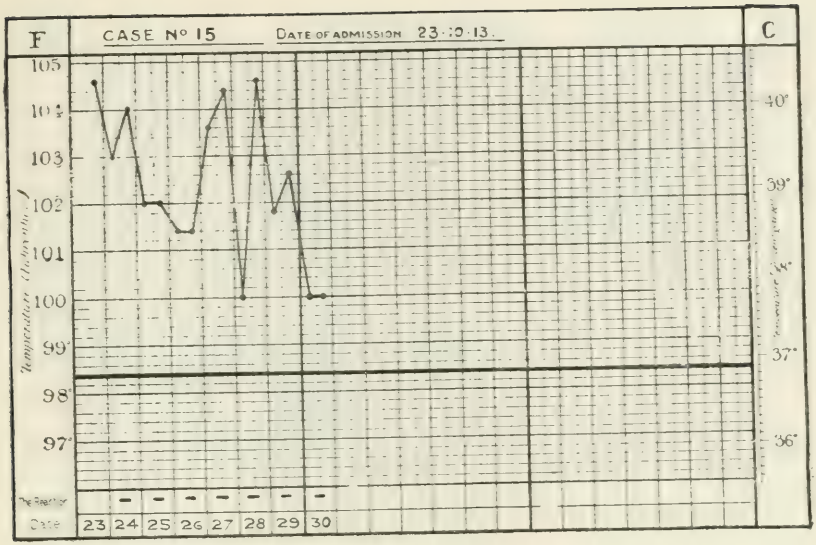

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DATE OF ADMISSION 11·10·13
CASE N°
DATE OF ADMISSION
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Temperature (Fahrenheit)
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Nov 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19

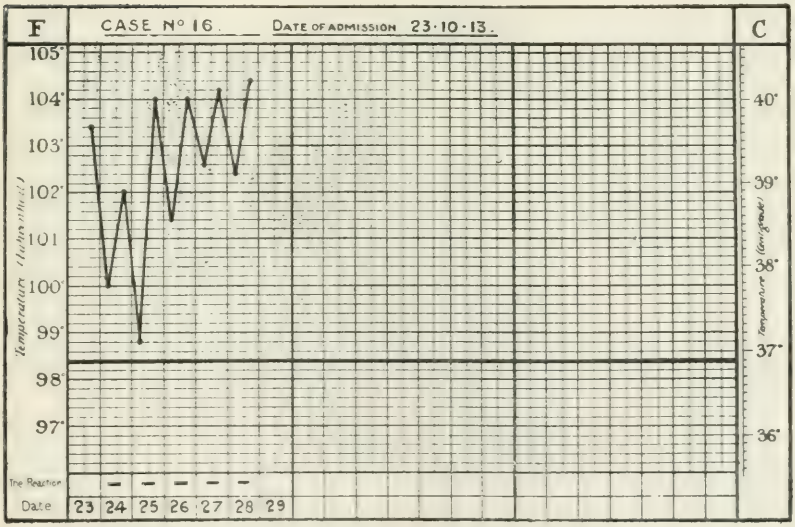

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CASE Nº 11.
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Temperature (Fahrenheit)
The Reaction
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Date
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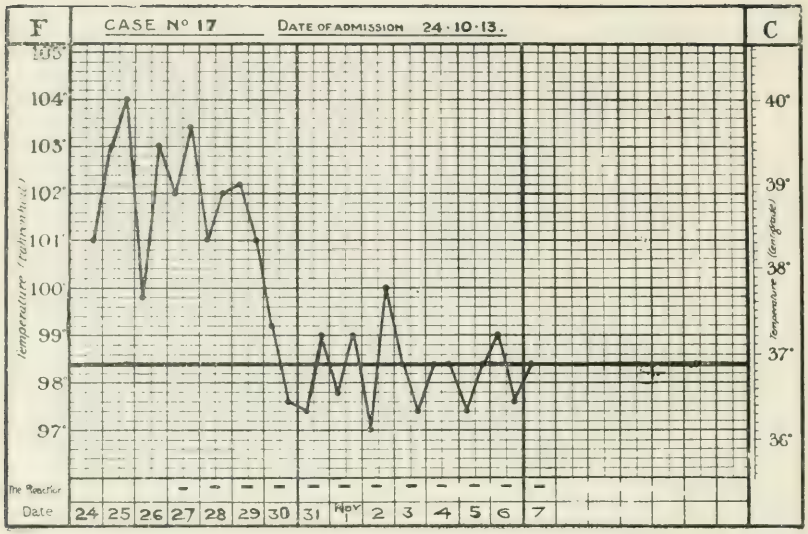

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CASE Nº 12
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Temperature (Fahrenheit)
The Reaction
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Date
14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31

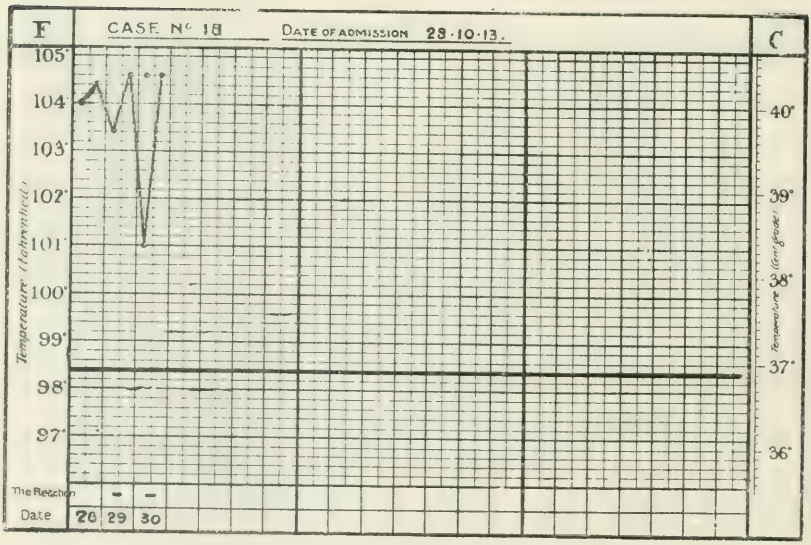

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Temperature (Fahrenheit)
Temperature (Centigrade)
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Date
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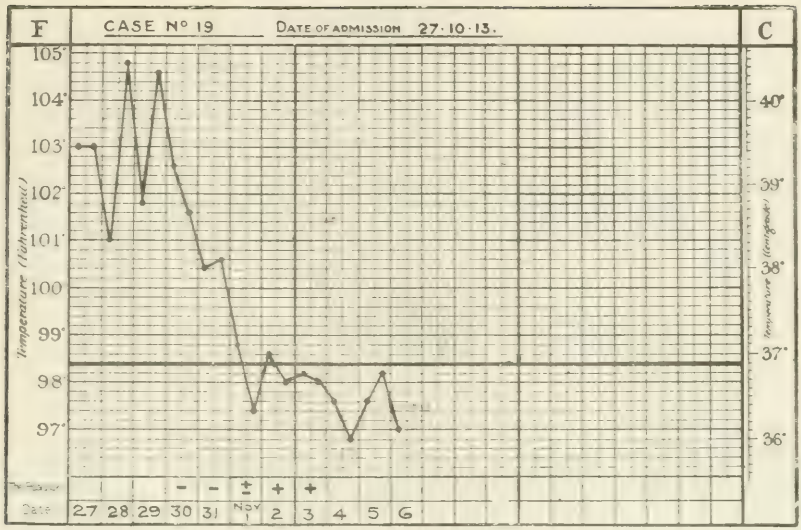

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Temperature (Fahrenheit)
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The Reaction
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Date
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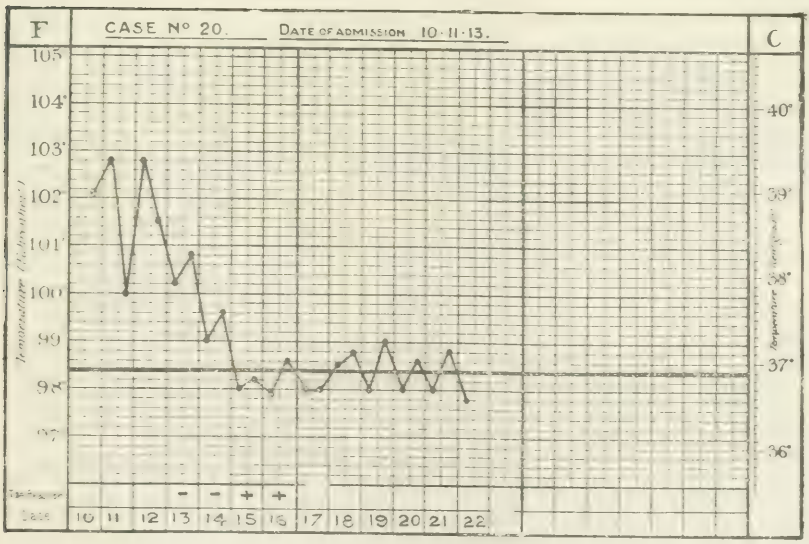

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40°
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36°
Temperature (Fahrenheit)
Temperature (Centigrade)
The Reaction
Date
23 24 25 26 27 28 29 30


F
CASE Nº 16
DATE OF ADMISSION 23·10·13.
C
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Temperature (Fahrenheit)
Temperature (Centigrade)
The Reaction
Date
23 24 25 26 27 28 29


F
CASE. N° 17
DATE OF ADMISSION 24·10·13.
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36°
Temperature (Fahrenheit)
Temperature (Centigrade)
The Reaction
Date
24 25 26 27 28 29 30 31 Nov 1 2 3 4 5 6 7


F
CASE. N° 18
DATE OF ADMISSION 28·10·13.
C
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Temperature (Fahrenheit)
Temperature (Centigrade)
The Reaction
Date
28 29 30


F
CASE Nº 19
DATE OF ADMISSION 27·10·13.
C
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36°
Temperature (Fahrenheit)
Temperature (Centigrade)
Pulse
Date
27 28 29 30 31 NOV 1 2 3 4 5 6


F
CASE Nº 20.
DATE OF ADMISSION 10·11·13.
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Temperature (Fahrenheit)
Temperature (Centigrade)
Pulse
Date
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Before proceeding to the experimental results it may be advisable to record here some previous observations on the quantity and composition of lung-ash.

West (v. supra) quotes Kussmaul as having found that the ash of the normal lung of infants contains no silica, but as age advances the amount of silica increases. He further found that in the case of quarrymen and men occupied in sandy trades the amount of silica rose to from 40 to 50 per cent. of the total ash.

Arlidge (Milroy Lectures, "Lancet," 1889, i., 615) quotes an analysis, by Church, of a potter's lung: Silica, 47·78 per cent.; Alumina, 18·63 per cent.; Iron oxide, 5·35 per cent., the remanider consisting of alkalis,phosphates, etc.

Oidtmann (v. Gorup-Besanez, Lehrbuch, 4th edition, 732) found that the lungs of a 14-day old child contained 19·82 per cent. of dry matter with 0·57 per cent. of mineral matter: the dry matter, therefore, contained 2·88 per cent. of ash.

Schmidt (ibid. 727) found the following constituents in the ash of normal human lung: 13·0% NaCl, 1·3% K_2O, 19·5% Na_2O, 1·9% CaO, 1·9% MgO, 3·2% Fe_2O_3, 48·5% P_2O_5, 0·8% SO_3, and 13·4% sand.

H. G. Wells (Chicago) in his "Chemical Pathology" (1907), in discussing Pneumonokoniosis, has collected the following data: Woskressensky (Cent. f. Path., 1898, 9, 296) found silicates in all of 54 lungs examined, except in two from infants. The lungs of individuals whose occupations do not expose them especially to dust inhalations contain increasing amounts of silicates in direct proportion to age; the silicates constitute from 3·5 to 10 per cent. of the total ash of the lungs. Thorel (Zieglers Beitr., 1896, 20, 85) reports that the lungs of a worker in soapstone were found to contain 3·25 per cent. of ash, including 2·43 per cent. of soapstone. Hodenpyl (Medical Record, 1889, 56, 942) obtained the following results with the lung of a knife-grinder who had followed his trade for about 15 years: Dry weight of lung, 48·1009 grams; total solids, 44·7986 grams. Composition of the ash: 27·1% SiO_2, 2·0% Fe_2O_3, 32·9% Al_2O_3, 2·2% CaO, 7·9% Na_2O, 4·9% K_2O, 21·4% P_2O_5, and 1·6% SO_3.

For the present investigation six lungs removed from the cadavers of miners who had suffered from silicosis were available. In all cases the right lung was taken for examination. For comparison, a precisely similar examination was made with the right lung removed from the cadaver of a male Zulu, aged 30, who had never worked underground and whose lungs presented entirely normal appearances on post-mortem examination: in the sequel this lung is referred to as "normal."

The procedure was as follows: On removal of the lung from the cadaver the wet weight was determined. The lung was then immersed in boiling water and the boiling was continued for some time. The first immersion in the hot water caused an immediate coagulation over the surface of the lung and so prevented anything but a negligible extraction of soluble salts. The lung was now minced and well dried in the steam-oven. The weight of the dry matter was then determined; no very great accuracy is claimed for this dry weight as no particular pains were taken to dry to constant weight.

Since the deposition of foreign mineral matter in the lung tissue is probably not uniform it was deemed advisable to ash the whole lung rather than risk the possibility of error in ashing only a portion which might not be representative of the whole. The ashing was carried out in a large platinum basin by first igniting over a free flame and subsequently burning off the carbon in a muffle furnace at a dull red heat.

The first table gives the results obtained, and in addition the amount of silica (calculated from the subsequent analysis): the weights are expressed in grams.

TABLE I.

Case	Normal.	No. 1.	No. 2.	No. 3.	No. 4.	No. 5.	No. 6.
Wet weight	649	907	1928	1672	1335	1497	1998
Dry weight	76	200	336	232	215	208·5	233·5
Ash	3·77	9·078	21·706	12·70	20·00	13·55	15·20
Silica	0·55	2·79	6·38	5·19	9·60	6·21	6·57
Ash: % of dry matter	4·96	4·54	6·46	5·47	9·30	6·49	6·51
Silica: % of ash	14·7	30·7	29·4	40·8	48·0	45·8	43·2
Silica: % of dry matter	0·73	1·39	1·90	2·23	4·47	2·98	2·81

The material described as "silica" is mainly silica, but may contain small amounts of very refractory silicates.

The weight of a healthy lung on removal from the body is extremely variable, but as a rough average a weight of 700 grams may be taken as normal for the right lung of an adult male. The high wet weights of the silicotic lungs are due, not entirely to the presence of a large amount of fibrous tissue, but also to their carrying an excess of serous fluid.

It will be observed that (1) the total weight of silica in the diseased lungs was much higher (from 2·8 to 9·6 grams) than in the normal lung (0·55 gram), and (2) the proportion of silica in the ash of the diseased lungs is much greater (from 29 to 48 per cent.) than in the normal lung (14·7 per cent.).

ANALYSIS OF THE ASH.

The ash from cases No. 1 and 2 was white, or nearly so; that from case No. 5 had a faint pink tinge; that from case No. 6 had a faint red tinge. The ash from the normal lung was slightly reddish, and the ashes from cases No. 3 and 4 were markedly red, corresponding with the comparatively high proportion of ferric oxide which they contained.

Ordinary mineral analysis of the ashes gave the following percentages of the constituents named:

TABLE II.

Case	Normal.	No. 1.	No. 2.	No. 3.	No. 4.	No. 5.	No. 6.
Silica	14·71	30·70	29·38	40·86	48·02	45·82	43·20
Ferric oxide	7·34		2·51	9·83	8·49	3·78	3·90
Alumina	5·47		5·50	7·71	9·59	7·90	7·72
Lime	10·98		5·55	3·64	2·16	2·45	4·40
Magnesia	2·66		1·54	1·47	1·64	1·62	1·41
Soda (Na_2O) ...	18·31		14·10	11·20	6·42	9·16	9·11
Potash (K_2O) ...	10·04		6·84	4·63	6·07	5·82	4·60
Phosphoric oxide (P_2O_5)	19·84		20·82	19·87	16·47	23·50	25·24
Sulphuric oxide (SO_3)	2·02		1·14	0·50	0·36	0·24	0·20
Chlorine				0·47	0·17		

It has already been pointed out (in connection with Table I.) that the amount of silica in the ash is greater in that from the silicotic lung than in that from the normal lung. From the fact that the lungs of infants contain no silica and the great probability that practically all the silica which accumulates in the lung during life gains access through inhalation of dust-laden air, it must be admitted that silica is a substance foreign to the lung. If this foreign substance be eliminated from the ash-analyses the following figures are obtained for the percentages of constituents in the ash-other-than-silica:

TABLE III.

Case	Normal.	No. 2.	No. 3.	No. 4.	No. 5.	No. 6.
Ferric oxide	8·6	3·5	16·6	16·3	7·0	6·9
Alumina	6·6	7·8	13·0	18·4	14·6	13·6
Lime	12·9	7·9	6·1	4·1	4·5	7·8
Magnesia	3·2	2·2	2·5	3·2	3·0	2·5
Soda	21·5	20·0	19·0	12·3	16·9	16·0
Potash	11·8	9·7	7·8	11·7	10·7	8·1
Phosphoric oxide	23·2	29·5	33·6	31·7	42·9	43·0
Sulphuric oxide	2·4	1·6	0·8	0·7	0·4	0·4
Chlorine			0·8	0·3		

Inspection of these figures shows that the amount of iron oxide varies irregularly, but the amount of alumina is increased in the silicotic lungs; this increase would be expected because alumina, in varying proportion, accompanies silica (as aluminium silicate) in the dust-laden air inhaled by underground workers. A noticeable feature of the results is the diminished proportion of lime in the diseased lungs as compared with the normal: the amount of magnesia is approximately constant. With regard to the alkalis there is indication of a slight diminution in the silicotic lung, but the decrease is not sufficient to be significant. The most striking fact which is brought to light by Table III. is the marked increase in the proportion of phosphoric oxide in the silicotic lungs: it is not improbable that this increase is associated with the great production of fibrous tissue which takes place (Watkins-Pitchford, *loc. cit.*, p. 46) as silicosis proceeds.

SIZE OF SILICA PARTICLES.

It is important to know the size of the silica particles which, inhaled in the dust, are deposited in the lung tissue. Dr. Watkins-Pitchford suggested that this should be ascertained by dissolving away the tissue in such a manner as to leave the silica particles untouched. In order to carry this out a portion of the left lung of case No. 5 was digested with hydrochloric acid and potassium chlorate until the tissue and all organic matter were destroyed. It is presumed that this process is entirely without action on the silica, and leaves it in its original condition; it may also leave some refractory silicates unchanged, but will dissolve practically all other inorganic matter. When the action was complete the residual white deposit was thoroughly washed by decantation so as to remove all soluble salts; care was taken that sedimentation was complete before decanting, and

this was aided by centrifuging the suspension. The white residue was then dried carefully at a low temperature so that no splintering of the silica particles should take place by excessive sudden heating. In this way about a gram of silica powder was obtained.

A smooth thin emulsion of the powder was made and submitted to microscopic examination. Examination with the polarising microscope proved that the material was silica.

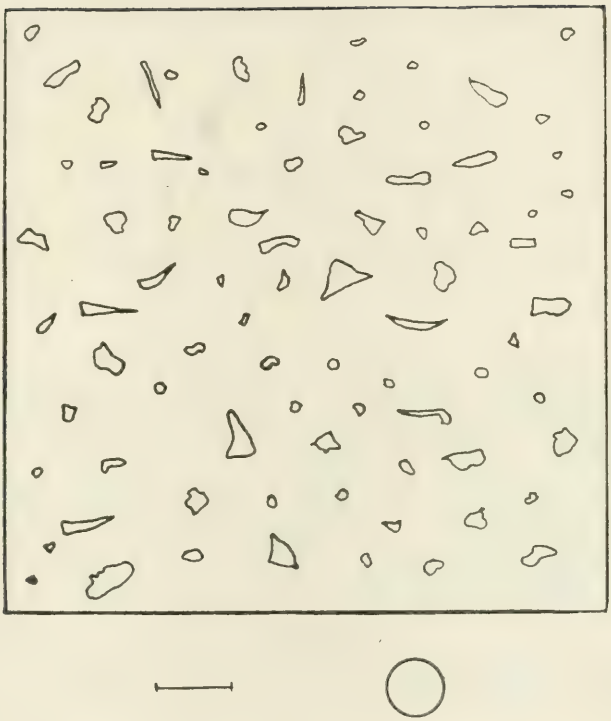
The great majority of the particles were found to be of indefinite shape and to have a diameter less than 1μ (that is, a diameter less than 1/8 of that of a red blood corpuscle); the amount of material with diameter less than 1μ was very approximately 70 per cent. of the whole. No attempt was made to measure diameters less than 1μ. Many measurements were made of the particles constituting the remaining 30 per cent. of the material: the longest diameters of these particles varied between 1μ and 8·5μ. In an extensive survey of several microscope preparations only a negligibly small number of particles was seen whose longest diameter exceeded 8·5μ, and the very longest observed was 10·5μ.

Watkins-Pitchford (*loc. cit.*, p. 47) states that the majority of the visible siliceous particles (as detected by means of polarised light) in sections of silicotic lungs vary from 2μ to 12μ in maximum diameter. Larger particles seen by Watkins-Pitchford in his histopathological investigation were found, not in the lung tissue, but in the cavities of bronchioles or alveoli.

The examination of silica particles isolated from this lung tissue (case No. 5) and the examination of silica particles *in situ* in lung tissue from several cases agree in leading to the conclusion that the largest particles which gain access to, and become embedded in, the lung proper have a maximum diameter of about 10μ (=about $\frac{1}{2500}$ inch): but few having a larger diameter (and these up to only 12μ) have been found in the lung.

The accompanying diagram represents the appearance presented by the larger particles of the material isolated from the lung: the 70 per cent. composed of particles with diameter less than 1μ has been omitted in the drawing. The diagram has been constructed to show characteristic shapes and their approximate frequency; it has not been constructed from a single field. It will be observed that a frequently occurring shape is a thin wedge, and particles with a less acutely triangular section are also prevalent. The most common shape, however, among particles of all sizes, is something approaching a sphere.

The line below the diagram represents 10μ on the same scale as the drawing, and the circle represents an average red blood corpuscle (nearly 8μ) on the same scale.



Sketch of larger particles of silica isolated from silicotic lung. × 1,000.

The line represents 10μ.

The circle represents a red blood corpuscle.

ANTHROPOLOGICAL NOTES ON BANTU NATIVES FROM PORTUGUESE EAST AFRICA.

By

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AND

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As we have had the opportunity of making a comparatively large number of anthropological records on certain Portuguese East African Natives, who had been recruited for work on the Gold, Diamond, and Coal Mines of the Transvaal, we thought it might be of interest to publish them, together with a few brief notes on the tribes concerned. The measurements were made on natives who had died before actually commencing work, as well as on those dying after having worked for periods of varying lengths. Both classes include natives who had come to the mines for the first time and also natives who may have been on the Rand on previous occasions.

With the exception of a few natives from the British Nyassaland Protectorate, all the cases referred to were recruited in Portuguese East Africa.

We are therefore dealing with a mixture of Bantu tribes, scattered over large tropical and sub-tropical areas. The attached map shows the various districts in which recruiting operations for the Transvaal mines have been carried on, and it will be noticed that the tropical and sub-tropical areas are divided by a well defined and substantial "buffer" district, namely, the Moçambique Company's territory. The whole area is bounded;—on the east by the Indian Ocean, on the north by the Rovuma River and German East Africa, on the south by Zululand, and on the west by Lake Nyassa, the British Nyassaland Protectorate, Rhodesia, the Transvaal, and Swaziland.

Owing to the enormous extent covered by recruiting operations, and the varying character of the country, our records include natives

of many tribes with different customs. The physical and climatic differences of their homes are also considerable, so that their diet and mode of living are subject to wide variations. Thus, for instance, this group includes, on the one hand, natives from high altitudes, such as the Lake Nyassa plateau, the Namuli peaks in Quilimane, and Angoniland north of Tete, in which places the altitude varies from three thousand to six thousand feet above sea level; and on the other, natives living in low lying areas, some of which are fertile, others swampy, and others again almost waterless and full of game. From these extremes in the character of the country we naturally find considerable variations in the habits, customs, diets, dwellings, and occupations of the inhabitants. Near the sea the people are, so to speak, amphibious, and live largely on fish; in other parts where water is scarce, they are hunters living on game, while in yet other parts the people are agriculturists and stock breeders. It must always be remembered, however, that owing to tribal wars, it is rarely that any one tribe exists for more than two generations (fifty years) in the same place and under the same conditions. Where one tribe predominates over another, it leaves its mark on the inferior race.

To attempt to go into a detailed account of the history of the groups would be outside the scope of this paper, and to classify them into tribes as if they were separate entities would be incorrect. We have, therefore, divided the natives into two main classes: (1) the sub-tropical, or as they are known on the mines, East Coast Natives, that is, Natives coming from between the Zulu border and Latitude 22° S.; and (2) Tropical Natives, or those coming from between the Zambesi River and German East Africa. These two groups are well separated from one another, and are composed of quite distinct types.

For the purpose of this paper, we have further sub-divided the sub-tropical or East Coast natives into Shangaans, or natives from Gazaland, Mytopis, Inhambaans, and Maputos or Tongas; and the tropical natives into, natives from Tete, Quilimanes, Moçambiques, and Portuguese Nyassas.

Before attempting to give details of these various groups, we would point out that the tribal wars previously mentioned, coupled with the fact that these wars often originated over the question of women, and certainly always terminated in the acquisition by the victorious tribe of all marriageable females, have brought about an extraordinary intermingling of race. Even when at peace, diplomatic exigencies frequently entailed the exchange of women between the various chiefs. Again, in early times, the results of the slave trade must have had a great effect on the intermingling of the tribes, and at a later date, the Portuguese policy of bringing black soldiers or

"cypaes"* from one colony to another, and of deporting chiefs and their followers from one part to another, has also had its effect in the same direction. In spite of the above-mentioned intermingling, the tribal peculiarities are still so marked as to make us hazard the suggestion that we believe we can, after our experience with natives, give a fairly accurate opinion as to the tribe of any native coming under our observation, and this from his general appearance. Of course in many cases an opinion is unconsciously formed from some tribal peculiarity or marking; however, we know from experience that it is not so easy to determine the tribe of a native when seen in the mortuary after death, so that probably a variety of scarcely recognised factors enter into the formation of one's judgment. It is remarkable that whereas Europeans are known to have cohabited freely with native women of the various tribes in tropical areas, yet the result in the way of half-castes is scarcely apparent.

It may be of interest to the reader unfamiliar with the movements of the native races, if we give a brief resumé of the recent history of the chief tribes dealt with in this paper.

The Shangaan. These people are an uncircumsised race of superior type to their neighbours. There are several accounts of their origin, and we think it probable, that while none of them are absolutely correct, elements of the truth are to be found in all. One story is as follows:—The Shangaans are descendants of impis sent north by Chaka; the best known of these was one which crossed the Zambesi, and eventually settled in the territory now known as British Central Africa, and from which the Angoni tribe of to-day originated. Some of these people eventually found their way into the country round Beira—old Gazaland. From this point they raided the neighbouring territories of Inhambane and Villainculos, driving out the original inhabitants, and forming small settlements throughout the districts. Spreading south they met the Mytopi, a fighting and stubborn race, who strenuously, and for many years resisted the invasion. Eventually, this warlike branch of the Zulu stock were victorious, and extended as far as the Limpopo River. Here they met the Portuguese, and were defeated by them near Chibuto, in 1896, their chief, Gungunyana, being captured and exiled.

Another version is as follows:—Umzilagazi, being sent north by Chaka, did not return to Zululand, but established himself in the Transvaal. He was driven out by the Boers by way of Umzilagazi's Nek in the Magaliesburg Mountains, and eventually established himself in Matabeleland. The remnants of his people are still to be

* English, Sepoy. French, Cipaye.

found round Middleburg; they call themselves Mandibile, and speak Zulu. From Matabeleland various raiding impis were despatched into the neighbouring territories. One under Soshangan went west and then south, until having had four leaders, namely, Soshangan, Manekuz, Muzila, and Gungunyana, it was conquered by the Portuguese in the present Gazaland, and Gungunyana deported.

It is interesting to note that the language of the "Abangoni"—who, according to this version, are an offshoot of Umzilagazi's troops, which went north of the Zambesi—is called "isi Nguni," and the Shangaans of the present day call the Zulu language "isi Nguni" in the same way as the Maxosa people call it "isi Chaka." It would appear that the present Shangaans last heard Zulu from the "Abangoni," and when they met it on their return south they called it "isi Nguni." The language of the Shangaans appears to have suffered a much greater deterioration from true Zulu than that of the Angonis.

There is still another version, namely, that Chaka sent an impi to the Limpopo, and that it was practically destroyed by fever, the remnant gradually spreading northward. Round the Limpopo there are still a few natives who say that they came from the south, and that their fathers died, not fighting, but in some catastrophe.

These sketches will serve to indicate how impossible it is to regard even a superior Bantu race as even approximately a pure stock. The women of the conquered races are always the perquisites of the victors. They further indicate the extreme difficulty of obtaining reliable historical accounts of native movements, even when confined to comparatively recent times.

The Mytopi. Although beaten by the Shaangaan they are still a virile race with distinct tribal peculiarities and customs. Formerly both males and females were tatooed with very distinct tribal markings, but of late years this practice has been almost entirely confined to the females.

The Inhambanes may, we think, be described as a heterogeneous collection of many tribes, harassed in turn by the slave-traders, the Shangaans, the Mytopis and others; they have never had a settled country, or attained any pre-eminence. Although living in a fertile and prolific country, they are of a somewhat inferior physique, and have been considered "dogs" by their more virile neighbours.

The Tongas, or natives of Maputaland, have been living for generations in the vicinity of Delagoa Bay, a port occupied for many generations by Europeans, Arabs, and Indians. They probably possess, therefore, more alien blood than any of the races already described. In addition, their close proximity to the Swazies has resulted in a

considerable intermixture with this race. The word Tonga implies "outcast," and as we see, they are a bastard race of, however, a somewhat higher degree of intelligence, and often of superior physique to that of their Bantu neighbours.

Natives from Tropical Areas.

The natives from the district of Tete consist largely of two main tribes, the Angoni and the Agawa. There are, however, a certain number of other, and, in our opinion, inferior tribes from the surrounding districts, which are comprised in this group.

The Angoni, as we have already stated, are of Zulu descent, being an offshoot of Umziligazi's force which settled in Matabeleland. They are a powerful, intelligent, and well built race, which has spread itself widely throughout Central Africa, some sections having spread as far to the north-east as German East Africa.

The Agawa (Yao) are, however, we consider, the finest race from these districts. Originally and principally slave-traders living in the hills surrounding Lake Nyassa, it was their custom to establish collecting stations on the eastern shores of the lake, to which slaves from the western side could be brought, and then handed over to the Arab dealers; in short, they were native middlemen in the slave trade of these parts. We believe they are, and have for long been, the predominant race, both mentally and physically. Many of them have embraced the Mohammedan religion.

We wish here to state definitely that our opinions in regard to the physical and mental characters of these natives are derived solely from the type of "boy" we have seen on the Rand, as neither of us has had an opportunity of visiting this part of Central Africa.

Natives from Portuguese Nyassaland vary considerably in type. Many have Swahili blood in their veins, while some appear to be practically pure Swahili. The Arab type of feature is very pronounced in a number of natives inhabiting the coastal belt of this district as well as of that further south. The Swahili language is understood by many, and serves as the *lingua franca* among the coastal natives of Nyassaland. While a large number of Nyassaland recruits come from the coastal districts, a smaller number are recruited from the interior, and are of a totally different type.

The Moçambique natives are a very heterogeneous class, but may be roughly sub-divided into four groups: the coastal native, the Makuas, the Lomwes, and the Parapatos.

The coastal native is of very mixed origin. Many show signs of Arab and Indian parentage, and not rarely have hair of an Asiatic character. The influence of the Portuguese custom of moving native troops from one part of the country to another, even bringing West

Coast troops to East Africa and *vice versa*, has left its mark in this as in other parts of the country. Traffic with the island of Moçambique, an old and important slaving centre and a modern port of some importance, has not been without its influence in this district. A large number of the natives profess the Mohammedan religion. Educationally and physically, the coastal native is in general of a better type than the native from the interior.

The Makuas are to be found in the north and north-eastern parts of this district. The name apparently denotes the wild or savage man, and no native acknowledges himself to be a Makua; they admit, however, that their language is the chi-makua. This group is sub-divided into many tribes, among which are Maravi's people, Nhamarals, Itoculos, Nacavala, Nacaroa, Moagems, and Irades. They all have deep incisions on the face, and are more or less tatooed on the body. The tribal markings vary considerably and are distinctive. Mr. Shepherd thinks that the Makuas are very old inhabitants, and in spite of the fact that their physique is better than that of any of the other inhabitants of the interior of this district, slave trading, tribal fighting, and small pox have prevented them from becoming a powerful race.

The Lomwes. These people inhabit the western and south-western parts of Moçambique. They came originally from the Lomwe, Higher, and Lower M'locue districts of Quilimane, spreading into the south-western part of Moçambique, which at that time was practically uninhabited. These people still maintain considerable inter-communication with the Lomwes across the Ligonha River. The Lomwes are much less tatooed than the Makuas. They are a wandering tribe, constantly on the move; this is due to a variety of causes, partly to the poverty of the land, which soon becomes exhausted when cultivated, partly to tribal feuds, and to avoid the inroads of the white man with its consequent taxation. Their language is very similar to that of the Lomwes of Quilimane, but contains many words which would not be understood by the latter. The Lomwes are physically a poor race. Being very lazy, and living in an unfertile country, they have frequently suffered from famine. Owing to these conditions they have not been able to obtain women from neighbouring tribes, and continual inbreeding has still further helped to deteriorate an already inferior race.

The Parapatos. This term includes the natives living in the district of Angoshe, a triangular area bounded, on the north by about the fifteenth parallel of latitude, and to the south by the Ligonha River. The most important tribe in this country are the Imbamellas. They are probably fairly old inhabitants, having affinities in language

and customs with the Lomwes, but are not tatooed to the same extent. The Arab influence among them has been considerable. They are tall, but otherwise not physically a fine race, and on the Rand show very little resistance to disease. They are chiefly engaged in agriculture, trading ground nuts to the Indian storekeepers; they are, however, lazy, and at times neglect their crops, so that they suffer unnecessarily from famine.

The district of Quilimane can be divided into four areas, namely:—Lomwe, Higher M'Locque, Lower M'Locque, and Maganja da Costa.

The natives in the Lomwe district are of poor physique. On two occasions in recent years they have been almost annihilated by famine and disease. They are exceedingly lazy, and will not take the trouble to plant crops, and even when this has been done, frequently neglect to harvest them. The inhabitants of Higher, and Lower M'Locque are very little superior to the Lomwes. Natives from the district of Maganja da Costa are a much finer race than those just referred to. They are a coastal tribe of superior physique and intelligence.

The above brief sketch of the various native races comprising the population dealt with in our records is not put forward with any claim to accuracy of detail, which it is exceedingly difficult to obtain even if at all possible. Our only object in touching on this matter here is to give the reader—unacquainted with the conditions among these races—some idea of the nature of the material, and to demonstrate the futility of attempting to regard any tribe as a pure strain even if only for a few generations. Nevertheless, there are very marked differences between the natives from tropical districts, and those coming from south of latitude 22° S., and when we come to sub-divide these groups, differences are still discernable though to a less extent. We reproduce drawings by Miss O. M. Vieich of native faces. These "boys" were chosen by Mr. Lloyd as typical of the race they represent; they further serve to illustrate the facial markings which are so noticeable a feature among many Bantu tribes.

We wish here to record our thanks; to Mr. B. G. Lloyd, General Manager of the Witwatersrand Native Labour Association, for reading through the manuscript of this part of our paper, and for giving us much valuable advice, and further, for allowing us to reproduce the drawings referred to above; and also to Mr. W. C. A. Shepherd, Chief Agent of the Witwatersrand Native Labour Association in Moçambique, for sending us notes on the natives of this district, from which we have quoted freely.

We can now turn to an examination of the actual records of the data obtained at the post-mortem examinations, on these natives, conducted in Johannesburg.

STATURE.

The measurements of stature, here considered, were obtained after death in the following manner:—The body being placed as straight as possible on the post-mortem table, the distance from the heel to the crown of the head was read off on a graduated staff. We had some misgiving as to whether this method might not introduce serious errors, owing possibly to post-mortem rigidity and to the unusual position of the body. These factors, however, if they be effective in appreciably altering the observed height from its true value would probably act in opposite directions; thus, muscular contractions would tend to diminish, and the dorsal-recumbent position, owing to a flattening of the spinal curves, to increase the apparent height. Judging from values found by other observers for living Bantu Natives, our post-mortem records do not suggest that any very large errors are introduced by this method of obtaining the measurement.

In Table I. the mean height in centimetres, the standard deviations, and coefficients of variation, are given for the different territorial classifications; the natives being grouped, as already explained, under headings according to the country from which they came.*

TABLE I.

Stature.

District.	Mean.	σ	C. of V.	N.
Moçambique	167·05 ± ·20	6·105 ± ·142	3·65	410
Quilimane	166·53 ± ·38	6·462 ± ·277	3·88	123
Port. Nyassaland	166·65 ± ·38	5·601 ± ·274	3·36	95
Tete	169·70 ± ·66	7·488 ± ·472	4·41	58
Total from Tropical Areas ...	167·16 ± ·15	6·269 ± ·114	3·75	693
Inhambane	168·99 ± ·51	6·599 ± ·363	3·91	75
M'chopi	171·22 ± ·53	5·255 ± ·381	3·07	43
Gazaland	170·49 ± ·56	6·597 ± ·404	3·87	61
Maputaland	166·83 ± 1·22	6·028 ± ·866	3·61	11
Total from East Coast	169·70 ± ·30	6·005 ± ·208	3·54	190
South lat. 22° S.	169·70 ± ·30	6·005 ± ·208	3·54	190

*Note.—In this and subsequent tables the numbers in the total groups do not always correspond with the additions of the numbers in the sub-groups, because of the inclusion of some cases which could not be classed under the sub-headings given, and were not sufficiently numerous to warrant the construction of further sub-groups.

It will be noticed that the Natives from South of Latitude 22° S. are taller and rather less variable in height than those from north of this parallel, and the question as to whether this difference may be regarded as a racial characteristic, or is due to the selection of the sample, must be considered. In the returns here dealt with, the Natives from Tropical Areas were mainly "new-boys," that is, "boys" detained in compound before allotment to the mines, while among those from the East Coast, the greater number had died while detained in compound on their way home, that is, most of them were being repatriated as being medically unfit, having usually some chronic disease, for example, tuberculosis. It is evident, therefore, that if there is any selective death-rate in regard to height, this point must be first investigated before conclusions as to the significance of the observed difference between these two groups can be drawn.

In Table II. the two main groups have been divided into, "new-boys" and "mine-boys," the former term applying to Natives who died while detained before allotment to the mines, and the latter to those who died while in compound after rejection by the mines as medically unfit.

TABLE II.

Stature and Disease.

Disease.	Mean.	σ	C. of V.	N.
Tropical Natives. "New-Boys."				
Tuberculosis	169·57 ± ·76	5·616 ± ·536	3·31	25
Intestinal Diseases ...	167·57 ± ·48	6·675 ± ·333	3·98	91
Cerebro-spinal-meningitis ...	167·44 ± ·33	6·975 ± ·231	4·17	207
Hydronephrosis	167·16 ± ·56	5·984 ± ·396	3·58	52
Pneumonia	167·14 ± ·25	6·533 ± ·173	3·91	322
Other medical causes ...	166·91 ± ·41	7·028 ± ·284	4·21	139
Total	167·29 ± ·15	6·698 ± ·112	4·00 ± ·07	836
Tropical Natives. "Mine-Boys."				
Tuberculosis	167·49 ± ·56	4·935 ± ·391	2·95	36
Other medical causes ...	168·00 ± ·56	5·474 ± ·404	3·26	42
Total	167·77 ± ·43	5·545 ± ·300	3·31 ± ·18	78
East Coast Natives. "New-Boys."				
Total	167·95 ± ·69	6·711 ± ·488	4·00 ± ·29	43
East Coast Natives. "Mine-Boys."				
Tuberculosis	170·13 ± ·41	6·025 ± ·287	3·54	100
Other medical causes ...	169·14 ± ·53	5·916 ± ·371	3·50	58
Total	169·78 ± ·33	6·005 ± ·229	3·54 ± ·13	158

In the group Tropical Natives "new-boys," no disease, with the exception of tuberculosis, is associated with any great variation in height from the mean. In the small group of deaths from tuberculosis here recorded, the mean height is over 2 c.m. above the average for the group. In this group there are, however, a few Natives who have worked on the Rand at some previous time, and are again coming up to work; it is among Natives in this class that tuberculosis is most frequently met with.[1] It is, we think, reasonable to suppose that this group therefore may be a selected group. Further, the coefficient of variability is rather smaller than that in any of the other sub-groups. In both of the main groups, the coefficient of variability is smaller among the "mine-boys" than among the "new-boys," while the mean height is greater. This could be explained on the assumption that there is a selective death-rate on the shorter "boy" in respect to acute diseases, in other words, the undersized and generally less developed subject is more prone to die from such diseases as cerebro-spinal-meningitis and pneumonia. It must not be forgotten, however, that there is apparently a racial difference in height, and that there is no doubt that there is a racial difference in liability to both the above-mentioned diseases. The highest pneumonia rate occurs amongst the Quilimane Natives, while the rate from cerebro-spinal-meningitis is highest among the Moçambique Natives, those from Nyassaland coming second, while the Quilimane Natives have a considerably lower mortality from this disease than either of the two former groups.

Considering these points in relation to the data here discussed, it seems fair to state that apparently there is some evidence in favour of the hypothesis that the death-rate may be selective in regard to stature, on the ground that the Natives who die after working on the mines—that is, after some months residence on the Reef—are not only taller than the newcomers, but the coefficient of variability of this measurement is smaller.

It will be necessary, therefore, to bear these facts in mind before any deductions are drawn from small differences obtained from these, and similarly selected classes. In regard to age, the bulk of the Natives considered may be classed as young adults; no Natives under eighteen and few of over fifty years are included in the returns.

Many observations among Europeans have shown that distributions of heights, when the data are drawn from a fairly homogeneous population, are very closely represented by the Normal or Gaussian Curve. In our figures the most homogeneous group of approximately sufficient size from which the type of the distribution could be calculated, was that of the Moçambiques. This group, however, is

not really homogeneous; the Natives from the Southern district, that of Angoche, being in many respects quite different from those of the Northern portion of this area. The constants obtained from the recorded height of the four hundred and ten Natives in Table I. is as follows:—

Mean	167·0548
σ	6·1050
β_1	0·0035
β_2	2·9966

$$y = 67 \cdot 633 e^{-x^2/74 \cdot 5420}$$

It is seen that this distribution conforms to the rule of normality mentioned above.

In Table III. a few mean heights from other races are given to enable the reader to compare the values with those just recorded. They have been selected from among the numerous published records, and their source acknowledged. We have also included some values for local native races compiled by the late Dr. Brodie, which we believe have not been published before. We have converted inches into centimetres when necessary, in order to facilitate comparison.

TABLE III.

Table of Heights.

3.	Cambridge Undergraduates. (1077) Galton	175·01
3.	British Middle Class. (1000) Pearson	172·83
3.	Galton S. Kensington Laboratory Series	172·47
5.	Bantu. Fritch	171·8
3.	New South Wales Criminals. (1000). Ages 25-30 ...	170·95
4.	Young Males. Swedes	170·18
4.	Young Males. Bohemians	169·42
4.	Young Males. Hessians	167·29
6.	East Coast Natives. (1337)	168·91
6.	British Basuto	168·91
5.	Bechuanas. Fritch	168·4
6.	Cape Colony. (680)	168·28
5.	American Negroes (pure). Gould	168·00
6.	Transvaal Basuto. (521)	167·64
6.	British Central African Natives. (121)	166·37
6.	Rhodesian Natives. (199)	166·37
6.	Bechuanas. (66)	166·37
6.	Zambesia Natives. (319)	163·20

* Reference number to bibliography at end of paper.

BRAIN WEIGHT.

The method of obtaining the brain weights was as follows: —The brain on removal from the skull was weighed to the nearest five grams without the removal of the pia-arachnoid. The pons, medulla, and cerebellum, were then separated, and the weight of the cerebrum alone noted. Nearly all the post-mortem examinations were conducted within twelve to twenty-four hours after death, the bodies being kept in the refrigerator pending examination. No deaths from accident are included in these returns, the cause of death in the majority of cases being some acute disease, for example, pneumonia.

TABLE IV.

Cerebrum Weights.

District.	Mean.	σ	C. of V.	N.
Moçambique	1136·1 ± 3·3	98·17 ± 2·31	8·64	410
Quilimane	1127·1 ± 5·6	92·40 ± 3·97	8·20	123
Port. Nyassaland	1128·6 ± 5·3	76·70 ± 3·75	6·80	95
Tete	1147·4 ± 8·8	99·60 ± 6·24	8·68	58
Total Tropical Natives ...	1134·7 ± 2·5	94·87 ± 1·72	8·36	693
Inhambane	1109·5 ± 6·7	85·55 ± 4·71	7·71	75
M'chopi	1195·7 ± 9·6	93·73 ± 6·82	8·55	43
Gazaland	1107·2 ± 7·1	81·78 ± 4·99	7·39	61
Maputaland	1098·9 ± 31·1	152·85 ± 21·98	13·91	11
Total East Coast Natives ...	1104·9 ± 4·7	95·74 ± 3·31	8·66	190

TABLE V.

Cerebellum, Pons, and Medulla.

District from where recruited.	Mean.	σ	C. of V.	N.
Moçambique	166·36 ± ·52	15·68 ± ·37	9·43	410
Quilimane	163·14 ± ·89	14·63 ± ·63	8·97	123
Nyassa	166·27 ± 1·13	16·34 ± ·80	9·83	95
Tete	172·02 ± 1·30	14·60 ± ·92	8·49	58
Total	166·27 ± ·40	15·72 ± ·28	9·45	693
Inhambane	168·42 ± 1·35	17·50 ± ·96	10·39	75
M'chopi	165·00 ± 1·87	17·92 ± 1·32	10·86	43
Gazaland	168·72 ± 1·62	18·90 ± 1·14	11·20	61
Maputaland	162·50 ± 4·13	17·30 ± 2·92	10·65	11
Total	167·54 ± ·90	18·32 ± ·64	10·93	190

As these values are obtained from Natives who died from disease, it is clear they cannot be regarded as random samples of tribal weights, unless there is no selective death-rate in respect of this character. The cause of death may also have some effect on brain-weight by altering the quantity of blood in the vessels, or of cerebro-spinal-fluid in the ventricles. In this connection we must not overlook the observations of J. B. Blakeman. In a paper written by him in conjunction with Dr. A. Lee and Professor Pearson some figures are given which suggest that when death was occasioned by chronic diseases, the brain weight was less than when an acute disease was the cause. They write: "It will thus be seen that, with the exception of the males over forty-six, the cause of death makes a very substantial difference in the average brain weight."[7] It will be noticed that our results as they stand do not confirm this result, but as will be seen, there may be special reasons for this apparent want of agreement. As our groups are unavoidably heterogeneous in character, a tribal constitutional liability to a disease might lead to an undue number of cases from one source being included in the returns; and if the particular group of Natives had a tribal difference in brain weight from that of the remainder of the group, a false estimate as to the relation of cause of death and brain-weight might result. That tribal differences in liability to pneumonia and cerebro-spinal-meningitis do occur has been shown in other publications.[2]

These figures comprise data from a similar source to that already dealt with under the heading "Stature," the reader will therefore realise that most of the Natives in the group "Tropical" died from acute disease before going to work on the mines, while in the East Coast group the larger number were old "mine-boys" among whom the more chronic complaints predominate.

In Table VI. the cerebrum weights are recorded and classed according to the cause of death for the four groups, "Tropical new-boys" and "mine-boys," East Coast "new-boys" and "mine-boys."

From this table it will be seen that in the group Tropical Natives "new-boys" that even excluding cases of death from cerebro-spinal-meningitis, the average cerebrum weight is rather higher than that found for the whole group in Table IV., while for the group "mine-boys" it is distinctly lower. We can attempt, by the method devised by Professor K. Pearson "On the Probability that two Independent Distributions of Frequency are really Samples from the same Population,"[8] to determine whether these differences are accidental, or should be regarded as significant.

TABLE VI.

Tropical Natives. "New-boys."

Cerebrum Weights.

Disease.	Mean.	σ	C. of V.	N.
Cerebro-spinal-meningitis ...	1166·98 ± 4·17	90·13 ± 2·95	7·72	212
Hydronephrosis	1164·75 ± 11·20	115·02 ± 7·95	9·88	48
Intestinal diseases	1146·66 ± 6·89	96·90 ± 4·87	8·45	90
Pneumonia	1138·75 ± 3·42	90·71 ± 2·42	7·97	320
Tuberculosis	1137·50 ± 12·13	88·10 ± 8·58	7·74	24
Other medical diseases ...	1132·8 ± 5·11	93·12 ± 6·31	8·22	151
Total	1147·04 ± 2·19	94·20 ± 1·55	8·21	845
Total less Cerebro-spinal-meningitis	1140·36 ± 2·54	94·59 ± 1·79	8·29	633

Tropical Natives. "Mine-boys."

Disease.	Mean.	σ	C. of V.	N.
Tuberculosis	1108·33 ± 10·88	96·77 ± 7·69	8·73	36
Total	1097·94 ± 7·85	99·41 ± 5·55	9·05	73

East Coast Natives. "New-boys."

Disease.	Mean.	σ	C. of V.	N.
Total	1150·00 ± 10·09	89·77 ± 7·14	7·81	36

East Coast Natives. "Mine-boys."

Disease.	Mean.	σ	C. of V.	N.
Tuberculosis	1095·16 ± 6·93	99·08 ± 4·90	9·05	93
Total	1104·05 ± 5·44	98·10 ± 3·85	8·39	148

The following values were obtained by grouping the weights into units of one hundred grams and calculating χ^2.

Tropical Natives.

"New-Boys" and "Mine-Boys."

$\chi^2 = 19·075$ $P = ·008$

East Coast Natives.

"New-Boys" and "Mine-Boys."

$\chi^2 = 4{\cdot}4058 \qquad P = {\cdot}495$

Tropical "Mine-Boys" with East Coast "Mine-Boys."

$\chi^2 = 8{\cdot}1106 \qquad P = {\cdot}231$

The number of cases in the groups "mine-boys" is unfortunately very small, nevertheless it will be noticed that among both Tropical and East Coast Natives, the "mine-boys" have a considerably lighter cerebrum than the "new-boys," and that the Tropical "mine-boys" agree more closely with the East Coast "mine-boys" than with Tropical "new-boys"; and further, that East Coast "new-boys" have actually a higher cerebrum weight than Tropical "new-boys," although in Table IV. it appeared that the East Coast group had the lighter cerebrum. It will be evident, therefore, that caution must be used before deducing any conclusions from the tribal differences shown in these tables.

It appears probable that at any rate among Tropical Natives, there is a difference in cerebrum weight according to whether the Native has worked on the mines before death or not; but whether this difference be due to the nature of the disease, to the effects of mine work, or is evidence of a selective death-rate on the "boys" who have the lightest cerebrum on the mines, or the newcomers who have the heaviest cerebrum, remains to be determined. There does not seem to be any definite evidence that an acute disease such as pneumonia tends to be associated with a brain of above the mean weight or that tuberculosis is associated with a particularly light brain. On the other hand, the intestinal diseases, which in this group are chiefly represented by dysentry, are apparently associated with a brain slightly above the average weight: this, if it be a real relationship, is the more remarkable, in that it is not likely that any increase in the weight is due to an additional amount of blood in the cerebral vessels; for this disease is accompanied by considerable loss of the body fluids. Having regard, however, to the size of the sample and its variability, too much stress must not be laid on this observation.

Although the "mine-boy" has apparently in both of the main groups a lighter cerebrum than the "boy" dying before being allotted to work, yet, as we have seen, he is on the average a taller "boy." If in the group of deaths among "mine-boys" here dealt with, the taller Native has the lighter brain, we might expect to find—bearing in mind the fact that in these groups the brain weights show larger coefficient of variation than among "new-boys"—that there is a lower coefficient of correlation for height and weight of cerebrum than among the "new-boys." Among all Tropical Natives we find the correlation of stature with weight of cerebrum is considerably larger than that among East Coast Natives (see Table VIII.), and as already mentioned, the former group contains very few "mine-boys," while in the latter the majority belong to this class. It will be necessary to collect larger numbers before any definite opinions can be formed in regard to a selective death-rate in respect to brain weight; nevertheless, these figures serve to point a warning, namely, that great care must be exercised before racial differences are asserted to exist from the examination of records of post-mortem weights, when the conditions under which the subjects were living prior to death were not comparable. While the cerebrum weight of the Tropical Native is apparently greater than that of the East Coast Native, there is no evidence that any similar variation occurs in regard to the weight of the cerebellum, pons, and medulla.

One curious feature is noticeable on reference to Tables IV. and V. At first sight we should hardly expect to find the coefficient of variability of the cerebellum, pons, and medulla, to be greater than that of the cerebrum, but in each of the above cases it is so. Dr. Raymond Pearl has drawn attention to the fact that the cerebrum is more variable than the whole brain; he writes: "The cerebral hemispheres are markedly more variable in weight as judged by the coefficient of variation than is the entire encephalon. This greater variation may denote a really greater variability of this part of the brain, or it may be due to the variable element which enters as the result of the separating of these organs from the rest of the brain. It seems likely to me that it is in part, at least, real for the following reasons: first, the cerebral hemispheres have attained relatively enormous development late in their phylogenetic history; and again from the functional standpoint the cerebrum is the most variable part of the brain."[9] We agree with Dr. Pearl in regard to his latter surmises, but as to the greater variability being possibly due to "a variable element which enters as a result of the separating of these organs," we think there would probably be considerably more variation produced by severing the medulla at different distances from the pons

than in separating the cerebrum by division of the crura. Further, the greater variability of the cerebellum, pons, and medulla, observed in our figures may, we think, probably be due to this variation in length of the medulla included in the specimens: clearly these organs will have two such sources of error, while the cerebrum can have but one.

The weight of the cerebrum is correlated with the weight of the rest of the encephalon, but our figures do not permit of any accurate measurement of this value for strictly homogeneous groups. The following table gives the correlation of cerebrum weight with that of the cerebellum, pons, and medulla.

TABLE VII.

Cerebrum with Cerebellum, Pons, and Medulla.

		Moçambique.	Total Tropical.	East Coast.
r	=	·4975 ± ·0250	·4604 ± ·0202	·3565 ± ·0428
$r\frac{\sigma_c}{\sigma_b}$	=	1·2451	1·1010	·7271
$r\frac{\sigma_b}{\sigma_c}$	=	·1988	·1910	·1748

Much has been written in regard to the relation of stature to brain weight, and a short consideration of this point will not be out of place. It has been claimed by some authors that there is a close agreement between height and brain weight. This may be true as regards tribal values for similar races, but recent researches prove that the correlation among individuals is in all cases only a low one. Our observations show that this is also the case in regard to these Bantu races, and that the correlation values are similar to those obtained from European data.

Dr. Raymond Pearl[10] gives the following values for the correlation of Brain-weight and Stature in the following groups, for young males:—

Swedes	·1830 ± ·0320
Hessians	·1823 ± ·0299
Bohemians ...	·2034 ± ·0397
Bavarians	·1664 ± ·0343

Cerebrum and Stature Eng. Males r = ·1202 ± ·0397.

Our values are as follows:—

TABLE VIII.

Stature with weight of Cerebrum.

		Moçambique.	Tropical Natives.	East Coast Natives.
r	=	·1415 ± ·0326	·1391 ± ·0251	·0402 ± ·0495
$r\frac{\sigma_c}{\sigma_s}$	=	·2329	·2131	·0651
$r\frac{\sigma_s}{\sigma_c}$	=	·0860	·0908	·0248

Stature with weight of Cerebellum, Pons, and Medulla.

		Moçambique.	Tropical Natives.	East Coast Natives.
r	=	·0957 ± ·0330	·1740 ± ·0248	·1065 ± ·0490
$r\frac{\sigma_c}{\sigma_s}$	=	·0634	·1094	·0784
$r\frac{\sigma_s}{\sigma_c}$	=	·1444	·2769	·1447

In both the latter groups the correlation of stature and cerebrum is lower than that of the rest of the brain and stature, the Moçambique group, however, is an apparent exception to this rule if it may be so termed. In regard to the cerebrum and stature value for Tropical Natives, this does not differ materially, having regard to the probable errors, from that found by Dr. Pearl for English Males. The value for the East Coast Natives is considerably below that for the Tropical Natives, and for English Males. We have already seen that, for some reason or other, among "mine-natives" the cerebrum weights are apparently less than that of Natives dying before allotment to the mines, and on the other hand these "mine-natives" may have a higher stature. If the explanation that selective death-rates in regard to these characters are taking place be correct, this may have some influence in lowering the correlation between these characters in this group; and the fact that there is little or no evidence of selection in regard to cerebellum, pons, and medulla weights, might account for the higher values found for these organs when correlated with stature. The following suggestion may be put forward as a possible explanation of this observation. The Native with the heavier brain and shorter stature conforms more closely to the European ratio of cerebrum weight to height, and he may therefore represent a higher type of development than the mean of his race, and thus be less stable, and also less resistant to abnormal conditions.

The ratio of stature in c.m.s. to cerebrum, and to cerebellum, pons, and medulla weights in grams for the various groups is shown below:—

TABLE IX.

Ratio of Weight, (1) Cerebrum, and (2) Cerebellum, Pons, and Medulla, to Stature.

Weight in Grams to Stature in Centimetres.

Country of Origin.	Cerebrum.	Cerebellum, Pons, and Medulla.
Moçambique	6·80	0·996
Quilimane	6·77	0·980
Nyassa	6·77	0·998
Tete	6·76	1·014
Total Tropical	6·79	0·994
Inhambane	6·56	0·997
M'chopi	6·40	0·964
Gazaland	6·49	0·990
Maputaland	6·59	0·974
East Coast Total	6·51	0·987

Marshall has given the ratio for Europeans of cerebrum in grams to height in centimetres as seven. If we accept this as the correct ratio for Europeans, it will be apparent that the ratios here recorded are of a rather lower value, and the cerebrum weights would have to be increased by about thirty-five grams for Tropicals, and eighty-six grams for East Coast Natives to bring the ratio to the value seven. From the table of weights of cerebrum and stature for English Males given by Dr. Pearl, and already referred to, we find the ratio to be 6·9, and presumably all adult ages may be included in this table. It has been shown by many writers on the subject that brain weight is negatively correlated with age after growth is complete. We might expect, therefore, that this ratio would show diminishing values as the mean age of the group was increased, unless the decrease in height with age occurred at a similar rate, which there is reason to doubt. Thus, there is some *a priori* reason for considering that the Bantu cerebrum is rather lighter in relation to stature than that of the European.

The following table gives the constants for the distribution of cerebrum weights for the Mocambique group:—

TABLE X

Mean	=	1136·41 ±3·26
σ	=	98·2325 ± 2·3085
β_1	=	·0189 ± ·0074
β_2	=	2·7780 ± ·1823
N	=	412

These values for the "betas" are not far from those required to give a normal distribution. The actual type is Pearson's Type I., and the mean is in advance of the mode. Having regard, however, to the probable errors, it would not be safe to conclude that this distribution is not in reality normal, particularly when we recollect that the group is not really homogeneous.

SKULL THICKNESS.

There is a general impression current that the Native's skull is thicker than that of the European. We have heard it urged that the native's immunity to the ill effects following head injuries is due to this cause. We have formed the opinion, after witnessing many post-mortems on natives and examining their skulls, that this is probably an incorrect view, so far at least as it applies to the races here dealt with. We are aware that general impressions of this kind are very apt to be misleading, and we merely record ours here without wishing to attach to it any undue value.

The difficulty of dealing with this subject in an accurate manner is considerable, and we have not been able to devise any method of recording the average thickness of a skull by one number. The thickness of individual skulls varies greatly even in closely adjacent portions. We thought, however, that tribal or racial differences might be discovered, if present, by taking a series of measurements at more or less fixed points and determining average values. The method of obtaining the measurements were as follows:—After the "skull-cap" had been removed, the thickness of the skull at the line of incision was determined by calipers at eight points, viz., (1) occipital protuberance, and (2) at about one and a half centimetres on either

side of the thick portion. The mean of these two readings was recorded. (3) The thinnest portion of the skull on each side under the temporal muscle lying within about two centimetres anterior to the external auditory foramen. These two measurements were added together, so that, to obtain the mean value for one side, the recorded measurements require to be divided by two. (4) The greatest depth of the frontal crest measured to the inner table of the skull, even when this did not occur on the line of incision; and (5) a value on each side of this eminence and distant about a centimetre from it (unless a frontal sinus was present at the level of removal of the "skull-cap," in which case the measurement was taken at the extremities of this cavity) and the mean of these two values recorded as the frontal thickness. We may here remark that the frontal sinuses of these natives, more particularly those from Tropical Areas, appear to be much larger than these spaces in the European, and often extend upwards from three to even six centimetres from the level of the supraorbital arch.

On Plate I. are shown three sections (actual size) of the anterior portion of the skull, cut at the usual level for removal of the skull-cap, as examples of the not uncommon development of the frontal sinuses. The photographs (Plate II.), which were taken by illuminating the skull from the interior, indicate the height to which the sinus extends. The sinus shown in figure 1 extends five c.m.s. from the line of incision. In all these specimens the bone appears to be absolutely normal; it does not exhibit any roughening of the surface or other pathological condition.

TABLE XI

Skull Thickness in Temporal Region.

Combined measurement of both sides.

Country of Origin.	Mean.	σ	C. of V.	N.
Moçambique	4·3604 ± ·0328	1·2074 ± ·232	27·69	616
Nyassa	4·2810 ± ·0739	1·2827 ± ·0523	29·96	137
Quilimane	4·4111 ± ·0969	1·3634 ± ·0667	30·91	90
Tete	3·6429 ± ·1513	1·1867 ± ·1070	32·58	28
Total Tropical	4·3301 ± ·0284	1·2425 ± ·0201	28·69	871
East Coast	3·7778 ± ·0733	1·1290 ± ·0518	29·88	108

TABLE XII.

Average Thickness of Skull at Side of Occipital Protuberance.

Country of Origin.	Mean.	σ	C. of V.	N.
Moçambique	6·1656 ± ·0353	1·3000 ± ·0250	21·08	616
Nyassa	6·1861 ± ·0749	1·3000 ± ·0530	21·01	137
Quilimane	6·3000 ± ·0894	1·2578 ± ·0615	19·97	90
Tete	6·6082 ± ·1291	1·0125 ± ·0913	15·32	28
Total Tropical	6·1855 ± ·0307	1·3440 ± ·0217	21·73	871
East Coast	5·8611 ± ·0911	1·4041 ± ·0644	23·96	108

TABLE XIII.

Average Thickness of Skull to Side of Frontal Crest.

Country of Origin.	Mean.	σ	C. of V.	N.
Moçambique	5·0214 ± ·0443	1·6507 ± ·0313	32·86	631
Nyassa	5·1087 ± ·0892	1·5531 ± ·0631	30·40	138
Quilimane	5·5899 ± ·1112	1·5553 ± ·0786	27·82	89
Tete	5·2778 ± ·1478	1·3147 ± ·1045	24·91	36
Total Tropical	5·1018 ± ·0366	1·6234 ± ·0259	31·82	894
East Coast	4·8894 ± ·0729	1·5588 ± ·0515	31·88	208

TABLE XIV.

Depth of Frontal Crest.

Country of Origin.	Mean.	σ	C. of V.	N.
Moçambique ...	11·6569 ± ·0876	3·2637 ± ·0620	28·00	631
Nyassa	11·9203 ± ·2027	3·5296 ± ·1433	29·61	138
Quilimane	11·6683 ± ·2198	3·0748 ± ·1555	26·35	89
Tete	10·9445 ± ·2391	2·1271 ± ·1691	19·44	36
Total Tropical ...	11·6801 ± ·0734	3·2567 ± ·0591	27·88	894
East Coast	10·4567 ± ·1368	2·9242 ± ·0967	27·96	208

TABLE XV.

Values of β_1 and β_2 in the Moçambique Group.

Region.	β_1	β_2
Temporal	0·1319 ± ·0381	2·7292 ± ·1052
Occipital	0·0585 ± ·0220	2·8859 ± ·1264
Frontal	0·6784 ± ·2189	3·9372 ± ·6608
Frontal Spine	0·2758 ± ·1054	3·2277 ± ·3105

In all cases the native from Tropical Areas appears to have a slightly thicker skull than the native from the East Coast, South of Latitude 22° S. The variability in the thickness is approximately the same for all measurements in the two groups. The occipital measurement is, however, slightly more constant than any of the other recorded values. In all the distributions for the Moçambique natives the type is that of Pearson's Type I., and the mean is in advance of the mode. Although the actual values of the "betas" lead to skew curves, the probable errors make us hesitate to suggest that if our population were homogeneous and the numbers larger, we should not find that these distributions would be well fitted by the normal curve of errors.

Having obtained the above values for native skulls, we thought it would be of interest to compare the results with similar measurements for Europeans. We were unable, however, to find any similar records, and so referred the matter to Dr. D. E. Derry, University College, London. We are much indebted to him for sending us a report on the "Thickness of the Human Skull," by Dr. R. J. Anderson, published in 1882. Unfortunately, the sites at which most of our measurements were taken do not correspond with those adopted by Dr. Anderson, and we regret that we were not aware of his work at the time when our measurements were first undertaken. Two of his measurements, however, appear to correspond approximately with ours, namely, "G. in the middle fossa, near the centre of the squamous part of the temporal bone," and "I. occipital at the middle of the inferior fossae." We have, therefore, obtained the mean value of his series for male skulls in these positions, and converting inches into millimeters the values are as follows:—G. = 5·63 and I. = 7·89. It will be seen that if these points are really comparable with our measurements at "Temporal Region" and "Side of Occipital Protruberance" the

native skull is appreciably thinner than that of the European. We do not consider that we can lay any stress on the comparison for the reasons given, but at any rate there is here no evidence to support the contention that the native skull is thicker than that of the European.

CEPHALIC AND CRANIAL INDICES.

It has only been possible for us to obtain head and skull, length and breadth, measurements in a comparatively few cases. Horizontal length and breadth measurements were taken before removal of the scalp, and subsequently on the skull, after its removal at approximately the same places. The analysis of the values obtained are given in Table XVI., the natives being classed into only two groups, "Tropical" and " East Coast."

TABLE XVI.

District of Origin.	Mean.	σ	C. of V.	N.
Skull Length.				
Tropical	186·14 ± ·26	6·1448 ± ·1846	3·30	252
East Coast	186·92 ± ·76	6·7757 ± ·5386	3·63	36
Head Length.				
Tropical	190·63 ± ·27	6·3500 ± ·1908	3·33	252
East Coast	192·08 ± ·75	6·7139 ± ·5337	3·50	36
Skull Breadth.				
Tropical	133·61 ± ·20	4·8167 ± ·1447	3·61	252
East Coast	132·81 ± ·61	5·4151 ± ·4304	4·08	36
Head Breadth.				
Tropical	139·19 ± ·20	4·6649 ± ·1401	3·35	252
East Coast	137·75 ± ·65	5·7657 ± ·4583	4·19	36

In the following table we have collected some of the measurements of skull length and breadth, and the cephalic index, for negroes, which have already been published.

TABLE XVII.

District or Tribe.	Length.	Breadth.	Cephalic Index.	N.
11. North of Africa	182·85	133·15	72·87	39
11. Algeria, Soudan, and West Coast...	185·04	135·20	73·28	54
12. Congo	177·78 ± ·62	138·52 ± ·48	77·99 ± ·25	50
12. Gaboon. (1864)	179·48 ± ·53	135·48 ± ·32	75·48 ± ·26	50
12. Gaboon. (1880)	182·33	138·22	75·86	18
12. Angoni	184·34	134·58	73·05	25
12. Kaffir	190·62	137·36	72·13	36
12. Zulu	184·15	137·05	74·27	19
12. Negro, Northern	182·85	133·15	72·87	39
Tropical Natives	186·14 ± ·26	133·61 ± ·20	73·01 ± ·12	252
East Coast Natives	186·92 ± ·76	132·81 ± ·61	71·53 ± ·38	36

In Table XVIII. we give the cephalic and cranial indices for the natives from the two main geographical areas, dividing those from tropical districts into sub-groups.

TABLE XVIII.

District of Origin.	Mean.	σ	C. of V.	N.
Cranial Index.				
Moçambique	71·65 ± ·15	2·8660 ± ·1091	4·00	157
Quilimane	71·18 ± ·47	3·6845 ± ·3321	5·18	28
Nyassaland	72·61 ± ·32	2·9000 ± ·2244	3·99	38
Tete, B.C.A.	71·98 ± ·35	2·7621 ± ·2446	3·84	29
Total Tropical	71·65 ± ·13 β_1 = ·0043	2·9951 ± ·0900 β_2 = 3·5362 ± ·7253	4·18	252
East Coast	70·35 ± ·37	3·1635 ± ·2587	4·50	36
Cephalic Index.				
Moçambique	72·86 ± ·14	2·6876 ± ·1023	3·69	157
Quilimane	72·61 ± ·44	3·4676 ± ·3125	4·78	28
Nyassaland	73·66 ± ·35	3·2406 ± ·2507	4·40	38
Tete, B.C.A.	73·16 ± ·30	2·3965 ± ·2122	3·28	29
Total Tropical	73·01 ± ·12 β_1 = ·0010	2·8319 ± ·0851 β_2 = 3·2912 ± ·4972	3·88	252
East Coast	71·53 ± ·38	3·2583 ± ·2665	4·56	36

It will be noticed that in regard to the distributions of Tropical Natives, both the distributions of the cranial and cephalic indices are, within the limits of the probable errors normal, and conform to the observations recorded by Miss Fawcett, who wrote: "With the series of skull measurements such as the present, which are long for the craniologist, if short for the statistician, we shall reach for most practical purposes adequate graphical representation of the frequency by using the normal curve of deviation: $y = y_0 e^{-x^2/(2\sigma^2)}$

The mean cephalic index is, among Tropical Natives, 1·36 units, and for East Coast Natives 1·18 units greater than the mean cranial index. The constants for the distribution of the difference of the two indices, including both Tropical and East Coast Natives in the series, are as follows:—

Cephalic Index minus Cranial Index.

Mean	=	1·2366 ± ·0524
σ	=	1·3210 ± ·0371
β_1	=	·0193 ± ·1412
β_2	=	3·2556 ± ·5000
N	=	288

Thus, while for the whole series of measurements the cephalic index is greater than the cranial index, this is not necessarily the case when individual examples are dealt with.

Table XVI. shows that the average allowance to be made for scalp thickness, is for length, for Tropical Natives 4·5 m.m., and for East Coats Natives 5·16 m.m., and for breadth, 5·58 and 4·94 respectively. These values are considerably smaller than those obtained by direct measurement of scalp thicknesses. The thicknesses given in Table XIX. were obtained by placing a finely graduated rule against the freshly cut edge of the scalp and reading off the thickness. By this method no compression was exercised which it was difficult to avoid if calipers were used; on the other hand, it is possible a certain amount of flattening and evertion of the edges took place, giving a reading rather too high. When using calipers to the head a considerable compression of the scalp may take place, and this may account for the discrepancy between these two sets of measurements.

SCALP MEASUREMENTS.

The records in regard to thickness of scalp for Tropical Natives have been tabulated for "new-boys" and "mine-boys" in order to determine whether the class which suffered most from the wasting diseases had thinner scalps than the new arrivals. It will be observed that in the group "mine-boys" the thickness in the case of both measurements is less than that in the other groups, and also that the variability is greater. The number in the latter group is unfortunately very small, nevertheless, there is some indication that wasting diseases may bring about a small reduction in scalp thickness. The measurements were taken approximately at the sites where the calipers would be applied when measuring head length and breadth.

TABLE XIX.

Thickness of Scalp.

Tropical Natives.

	"New-boys."		*"Mine-boys."*	
	Temporal measurement. Two sides added together.	Frontal plus Occipital measurement.	Temporal measurement. Two sides added together.	Frontal plus Occipital measurement.
Mean	7·44 ± ·05	7·71 ± ·05	6·76 ± ·27	6·91 ± ·32
σ	1·8986 ± ·0341	1·9543 ± ·035	2·0452 ± ·1877	2·4665 ± ·2264
C. of V.	25·52	25·35	30·25	35·69
N.	704	704	27	27

Total East Coast Natives.

Mean	7·06 ± ·13	6·73 ± ·12
σ	1·9717 ± ·0917	1·8128 ± ·0828
C. of V.	27·93	26·94
N.	109	109

HEART WEIGHTS.

In considering the question of heart weights among the Natives comprised in our returns, an important point arises in connection with the change of residence which these "boys" undergo when arriving on the Rand. Many of them come from coastal and low lying districts, and are rapidly transferred to an altitude of nearly six thousand feet. It is usually assumed that a compensatory hypertrophy of the heart

takes place as a consequence; the hypothesis being, that owing to the decreased amount of oxygen per volume of air, more work must be undertaken by the heart. That this is so, has not, we consider, been definitely proved. It has been established that an increase in the number of erythrocytes occurs when an individual resides at an altitude, and that the viscosity of the blood is thereby increased; but whether this increased viscosity is compensated for by the greater oxygen absorbing efficiency of the blood, or whether there is still a balance of excess of work for the heart, has not yet, we think, been finally settled. If, however, when living at an altitude the heart is called upon for a greater amount of work (after acclimatisation has taken place) then it would seem reasonable to suppose that the normal heart would show signs of hypertrophy, and that we should find the average weight of hearts collected from post-mortem records of people dying after a period of residence at an altitude, was greater than among similar records from a coastal community.

Assuming the hypothesis that residence at an altitude produces cardiac hypertrophy to be correct, it would appear reasonable to expect that "mine-boys" would on the average have heavier hearts than "new-boys," for in addition to the fact that this latter class have only resided on the Reef for a few days, or at most a week or two before death, they also have not been doing the hard physical work to which the "mine-boy" has become accustomed. Reference to Table XX. will show that the figures recorded here do not support this assumption. Among "Tropical Natives" the "mine-boy" has an appreciably lighter heart than the fresh arrival, while among East Coast Natives, in this respect, there is no apparent difference in the two classes. The "mine-boys" in both groups, Tropical and East Coast, show greater variability in regard to heart weight than the "new-boys."

It cannot be assumed, however, that this observation proves the contention, that residence at an altitude is associated with hypertrophy of the heart, is unfounded, for the disease causing death is a factor which cannot be ignored. There are other possible factors concerned; for instance, it is conceivable that natives who have abnormally small hearts, or who have not the power to produce a compensatory hypertrophy, are more prone to develop chronic diseases; and thus an undue proportion of small hearts will appear in the mortality statistics of "mine-boys"; in other words, there is a selective death-rate in respect to the undersized heart. It has been contended that the tuberculous subject has on the average a smaller heart than the normal, but we do not know on what evidence this statement is made. Atheromatous degeneration of the arteries is very

rarely seen among these natives: and we are informed that it is also uncommon among European residents.* If it be true that cardiac strain is a factor in the production of this disease, its rarity may have some interest in this connection.

The constants for the distribution of heart weights for the four classes referred to, grouped according to the cause of death, are given in the table below. In cases where the heart itself was obviously diseased, that is where pericarditis or endocarditis was present, the weights have not been included when calculating these values: that is to say, only apparently healthy hearts have been included.

TABLE XX.

Heart Weights.

Tropical Natives. "New-boys."

Disease.	Mean.	σ	C. of V.	N.
Intestinal	237·32 ± 3·19	42·79 ± 2·25	18·03	82
Tuberculosis	253·08 ± 7·82	59·11 ± 5·53	23·36	26
Other Diseases (excluding Heart Disease)	256·85 ± 3·10	51·21 ± 2·19	19·94	124
Hydro-nephrosis	260·58 ± 5·23	55·91 ± 3·70	21·46	52
Cerebro-spinal-meningitis ...	283·12 ± 2·40	51·96 ± 1·69	18·35	214
Pneumonia	285·44 ± 2·16	56·72 ± 1·53	19·87	313
Total	273·04 ± 1·33	56·06 ± 0·94	20·53	811

Tropical Natives. "Mine-boys."

Tuberculosis	253·40 ± 8·84	65·51 ± 6·25	25·85	25
Total	252·85 ± 5·22	62·38 ± 3·69	24·67	65

East Coast Natives. "New-boys."

Total	259·68 ± 4·94	50·24 ± 3·50	19·35	47

East Coast Natives. "Mine-boys."

Tuberculosis	249·34 ± 4·84	62·62 ± 3·43	25·11	76
Total	260·00 ± 4·01	70·83 ± 2·83	27·24	142

* Glaucoma, another disease frequently associated with high arterial tension, is, we are informed by Dr. Napier, much less common on the high veld than at the coast.

In the following table the heart weights recorded by Dr. M. Greenwood in his paper on Weights of Human Viscera[13] are given; ounces have been converted into grams for the convenience of comparison.

TABLE XXI.

General Hospital Population	383·57—369·68
Apparently healthy organs	318·94—312·98
Cases of Pneumonia	354
Cases of Valvula Disease and Aortic Aneurism ...	541

It is doubtful how far these figures are comparable with those in Table XX. Greenwood has shown that there is a sensible correlation of heart weight with age, and it is probable that his figures were derived from cases with a higher average age at death than ours. Nevertheless the figures suggest that the mean heart weight of the European is heavier than that of these Natives. Unfortunately we have not been able to obtain records of heart weights of Europeans dying in Johannesburg.

The distribution of heart weights is apparently slightly skew and leptokurtic. The value of the "betas" for the group "Tropicals" pneumonia, and "All Tropicals," is as under. It will be seen that the arithmetic mean is a fairly close approximation to the modal values of the distributions.

Hearts from cases of	β_1	β_2
Pneumonia (among Tropical Natives)	$\cdot1351 \pm \cdot0295$	$3\cdot4564 \pm \cdot1386$
All Tropical Natives, excluding deaths from heart disease	$\cdot1676 \pm \cdot0185$	$3\cdot4136 \pm \cdot0801$

SPLEEN WEIGHTS.

The question of the average spleen weights of residents in tropical and malarial regions is of some importance. It is generally believed that inhabitants of these districts have larger spleens than residents in non-malarious countries. The splenic enlargement is especially marked in children, and the use of the "splenic index" as a measure of the prevalence of malaria, is generally assumed to be practically of little value unless confined to children.

Among the Natives here dealt with the spleen weight is exceedingly variable, and the distribution very skew, so that the mean weight does not approximate to the modal or commonest weight; and,

therefore, if used in the sense of commonest weight will be quite misleading. The distribution of spleen weights cannot be fitted by a single Pearson curve. For " Tropical Natives " the constants lead to a J shaped curve with the start at about one hundred grams; this is clearly an incorrect value, and the unsatisfactory fit is, we consider, due to the heterogeneous nature of the material, owing to racial factors and cause of death. Dr. Greenwood's figures show similar irregularities, but not to the same extent.[13] For purposes of comparison we have combined two of his tables of spleen weights, namely, 358 cases, ages 25-35, and 536 with ages 35-45. The constants of these 894 spleen weights are: Mean 196·75 grams, $\sigma = 101·7323$, C. of V. 51·71, $\beta_1 = 1·6770$, $\beta_2 = 4·7421$, Mode 87·03. In this case, judging from the crude distribution, we might expect the modal value to be somewhere in the neighbourhood of 142 grams. The variability of these spleen weights is about ten per cent. less than that obtained from our returns.

TABLE XXII.

Spleen Weights.

Tropical Natives.

District.	Mean.	σ	C. of V.	N.
Moçambique	267·01 ± 4·63	162·46 ± 3·27	60·84	560
Quilimane	235·21 ± 7·51	132·63 ± 5·31	56·39	142
Nyassaland	295·72 ± 7·84	139·48 ± 5·54	47·17	144
Tete	332·04 ± 13·85	173·06 ± 9·80	52·12	71
Total Tropical	267·04 ± 3·50	157·33 ± 2·48	58·92	917

East Coast Natives.

Inhambane	368·33 ± 19·11	268·76 ± 13·51	72·97	90
M'chopi	316·86 ± 16·37	159·19 ± 11·58	50·24	43
Gazaland	399·61 ± 20·20	237·69 ± 14·28	59·48	63
Maputaland	320·84 ± 20·44	105·00 ± 14·46	32·73	12
Total East Coast	374·02 ± 11·19	239·15 ± 7·91	63·94	208

It will be noticed that the mean spleen weight of Tropical Natives is considerably higher than that of Europeans, as judged by Dr. Greenwood's figures, and that the East Coast Native has a still higher average weight. We are unable to suggest any reason for the fact that the East Coast figures show an increase in weight of forty-three per

cent. over that obtained from the Tropical Natives. The spleen weight from cases of tuberculosis is apparently higher than that obtained when other diseases were the cause of death, and there is a greater percentage of deaths from this cause among East Coast Natives, but the following table shows that the spleen weight of tuberculous East Coast cases is thirty per cent. heavier than that of Tropical Natives dying from the same disease, and a similar difference exists for other medical diseases.

The value of the "betas" for the group "All Tropical Natives" are, $\beta_1 = 3{\cdot}9347$, $\beta_2 = 8{\cdot}6664$.

TABLE XXIII.

Spleen weight in respect to cause of death.

Tropical Natives.

Disease.	Mean.	σ	C. of V.	N.
Tuberculosis	318·44 ± 16·99	196·76 ± 12·02	61·79	61
Pneumonia	280·48 ± 5·37	148·33 ± 3·80	53·88	347
Cerebro-spinal-meningitis...	266·28 ± 6·55	141·45 ± 4·63	53·12	212
Other medical causes ...	251·03 ± 7·28	159·72 ± 5·15	64·58	219
Intestinal diseases ...	204·87 ± 8·72	113·47 ± 6·17	55·39	77
East Coast Natives.				
Tuberculosis	410·72 ± 18·07	274·53 ± 12·78	66·84	105
Other medical causes ...	322·58 ± 12·89	193·89 ± 9·11	60·11	103

LIVER WEIGHTS.

The native from Portuguese territory is very constantly infected with bilharziosis, and it is not uncommon to find the embryos of the schistosomum hæmatobium in the liver on post-mortem examination. It appeared possible that the presence of this parasite might have some effect in increasing the weight of this organ, as it has been stated that pathological changes accompany their presence in the liver. To determine whether an increase of weight was associated with its presence, five hundred and seventy-nine liver weights of Tropical Natives were classified according to whether the parasite had been observed or not, with the following result.

TABLE XXIV.

Liver weights in relation to bilharziosis.

Tropical Natives.

	Mean.	σ	C. of V.	N.
Embryos present	1438·16 + 10·34	267·40 + 7·31	18·59	304
Embryos not found ...	1395·45 + 10·46	257·27 + 7·40	18·44	275

It will be seen that while the livers in which embryos have been found were rather heavier than the others, the difference can hardly be considered significant, and it seems unlikely that the frequency of this disease will materially affect the average liver weights in these groups.

TABLE XXV.

Liver Weights.

Territory.	Mean.	σ	C. of V.	N.
Tropical Natives.				
Moçambique and Nyassaland ...	1417·88 ± 7·36	262·74 ± 5·21	18·53	579
Quilimane	1480·37 ± 18·59	320·25 ± 13·15	21·63	135
Tete	1535·42 ± 31·18	257·38 ± 22·05	17·94	48
Total Tropical	1437·50 ± 6·84	277·92 ± 4·83	19·33	752
East Coast Natives.				
Gazaland	1550·00 ± 28·68	272·30 ± 20·28	17·57	41
M'chopi and Inhambane ...	1530·65 ± 20·75	296·67 ± 14·67	19·38	93
Total East Coast	1532·52 ± 16·23	287·82 ± 11·48	18·78	134

The other constants for the distribution of Total Tropical weights are, Mode 1357·23, β_1 ·5044 ± ·1537, β_2 4·1678 ± ·6224.

KIDNEY WEIGHTS.

Hydronephrosis of the kidney is not an uncommon condition of the Tropical and East Coast Native. This is due to the thickening and occlusion of the ureter due to bilharziosis. Such kidneys have been omitted from the table of weights given below.

TABLE XXVI.

Kidney Weights.

	Mean.	σ	C. of V.	N.
Tropical Natives	267·50 ± 1·29	58·37 ± 0·91	21·82	936
East Coast Natives	279·90 ± 2·92	59·93 ± 2·07	21·41	191
All Natives	269·60 ± 1·16	57·48 ± 0·82	21·32	1127

Mode 249·24, β_1 1·462 ± ·3838, β_2 5·8502 ± 2·3708.

INTESTINAL MEASUREMENTS.

TABLE XXVII.

Small Intestine.

	Mean.	σ	C. of V.	N.
Tropical Natives	736·28 ±2·76	109·16 ±1·95	14·83	713
East Coast Natives	678·93 ±8·20	128·13 ±5·80	18·87	111
Large Intestine.				
Tropical Natives	162·93 ±0·62	25·00 ±0·44	15·34	734
East Coast Natives	159·18 ±1·88	29·05 ±1·33	18·25	109
Appendix.				
Tropical Natives	11·54 ±0·06	2·47 ±0·04	21·40	734
East Coast Natives	11·74 ±0·18	2·75 ±0·13	23·42	105

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PORTUGUESE EAST AFRICA.

Showing recruiting areas of W.N.L. Association, Ltd.

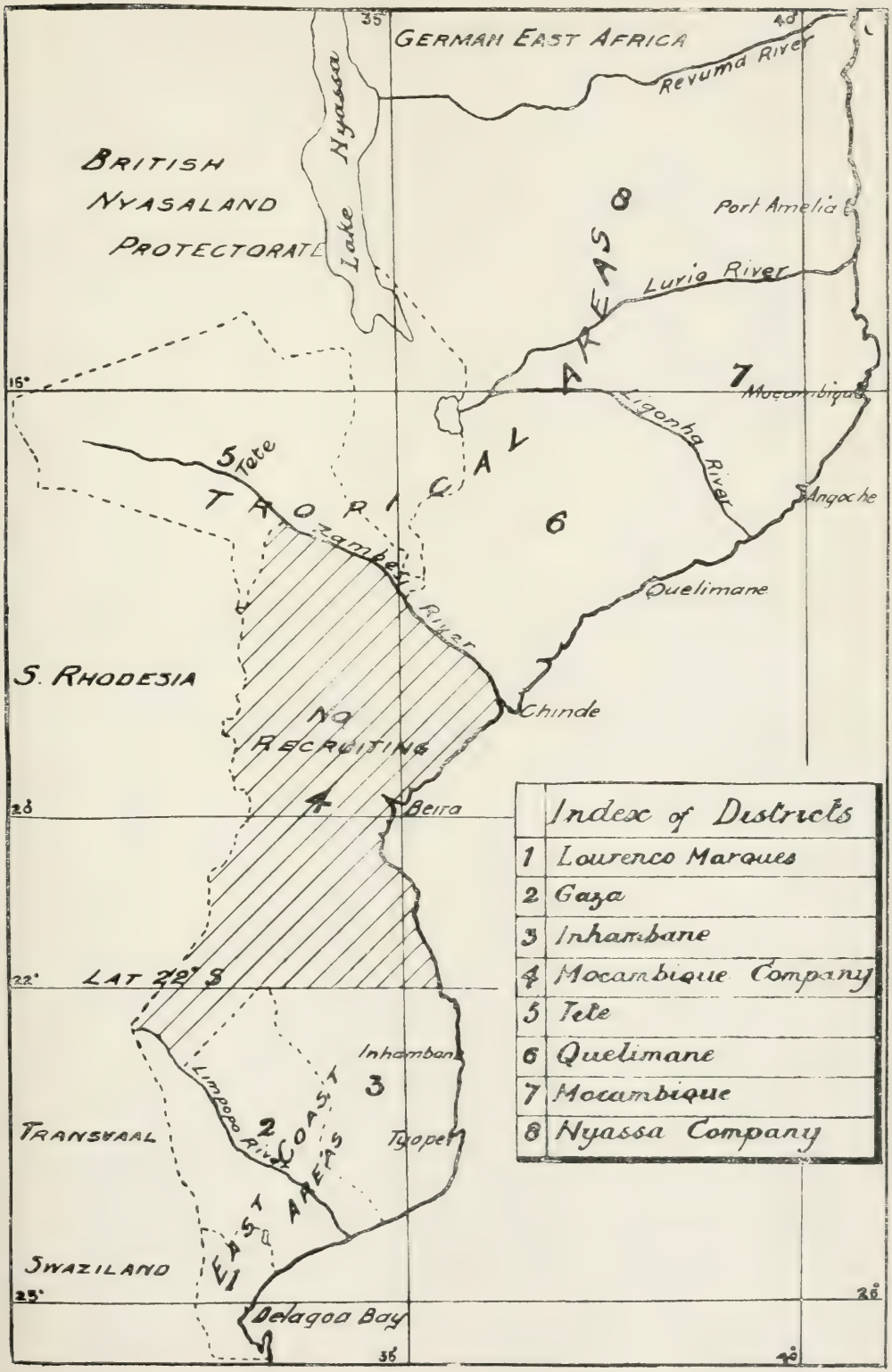


PLATE I.

Sections through skull showing frontal sinuses at line of incision for removal of skull cap.

(Actual size.)

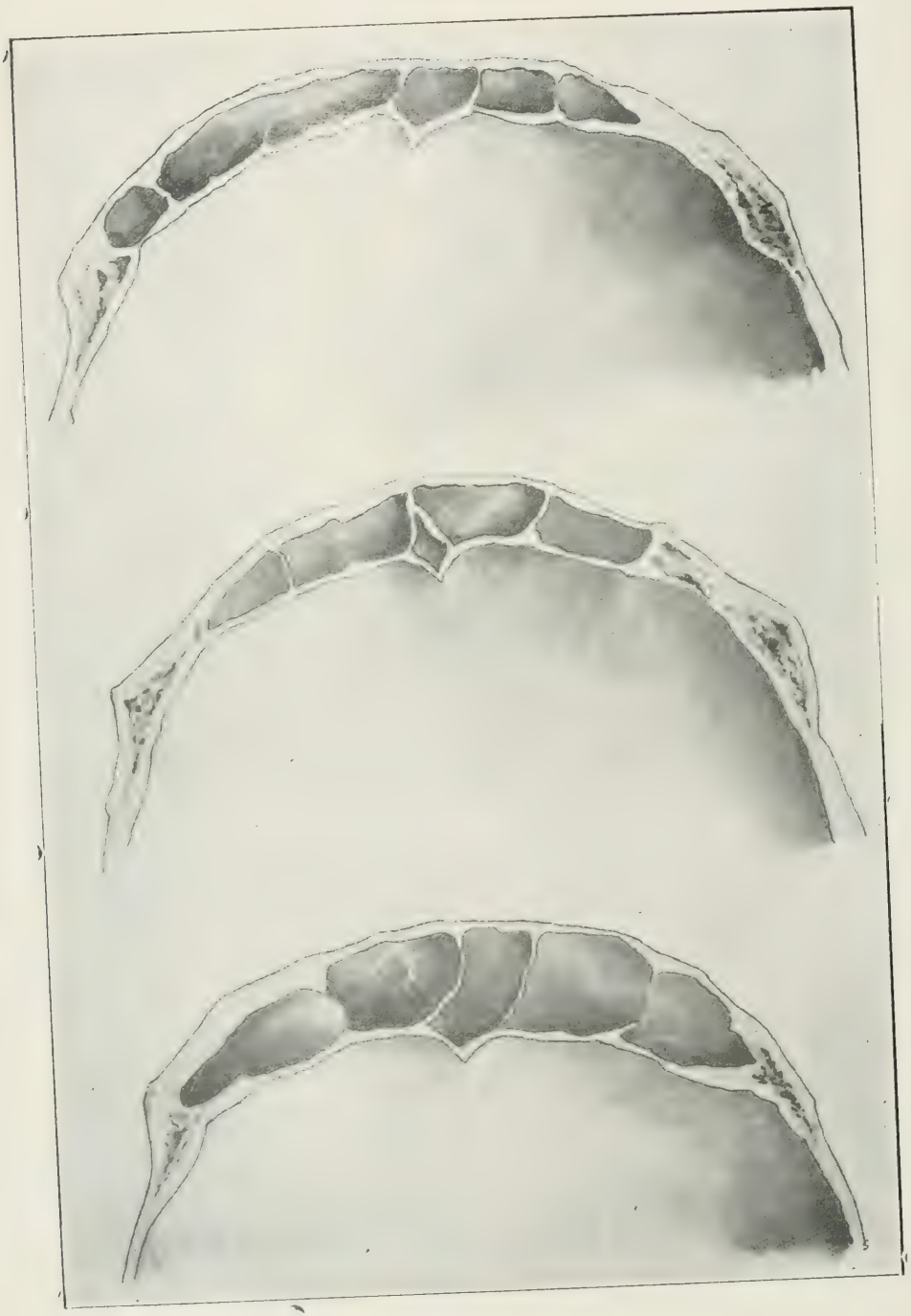
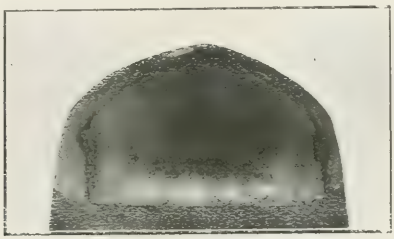
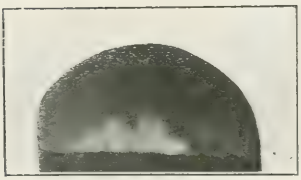
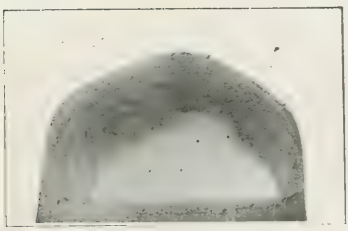


PLATE II.

Photographs showing development of frontal sinuses, corresponding to sections in Plate I.









Shangaan.



Shangaan (from Senna).



Tyopi.



Makua - Quilimane.



Makua—Quilimane.



Makua — Moçambique.



Makua—Moçambique.



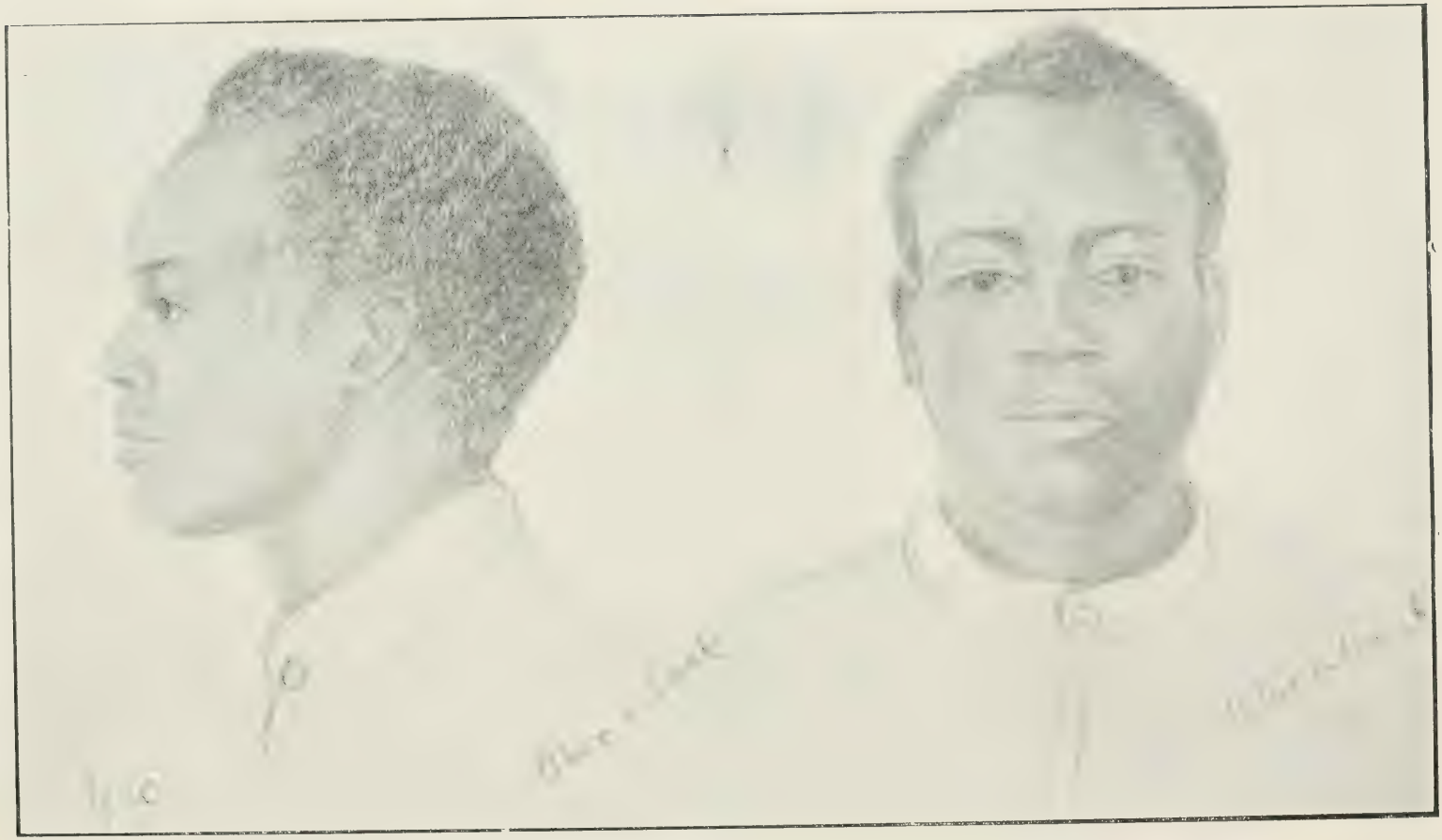
Makua — Mocambique.



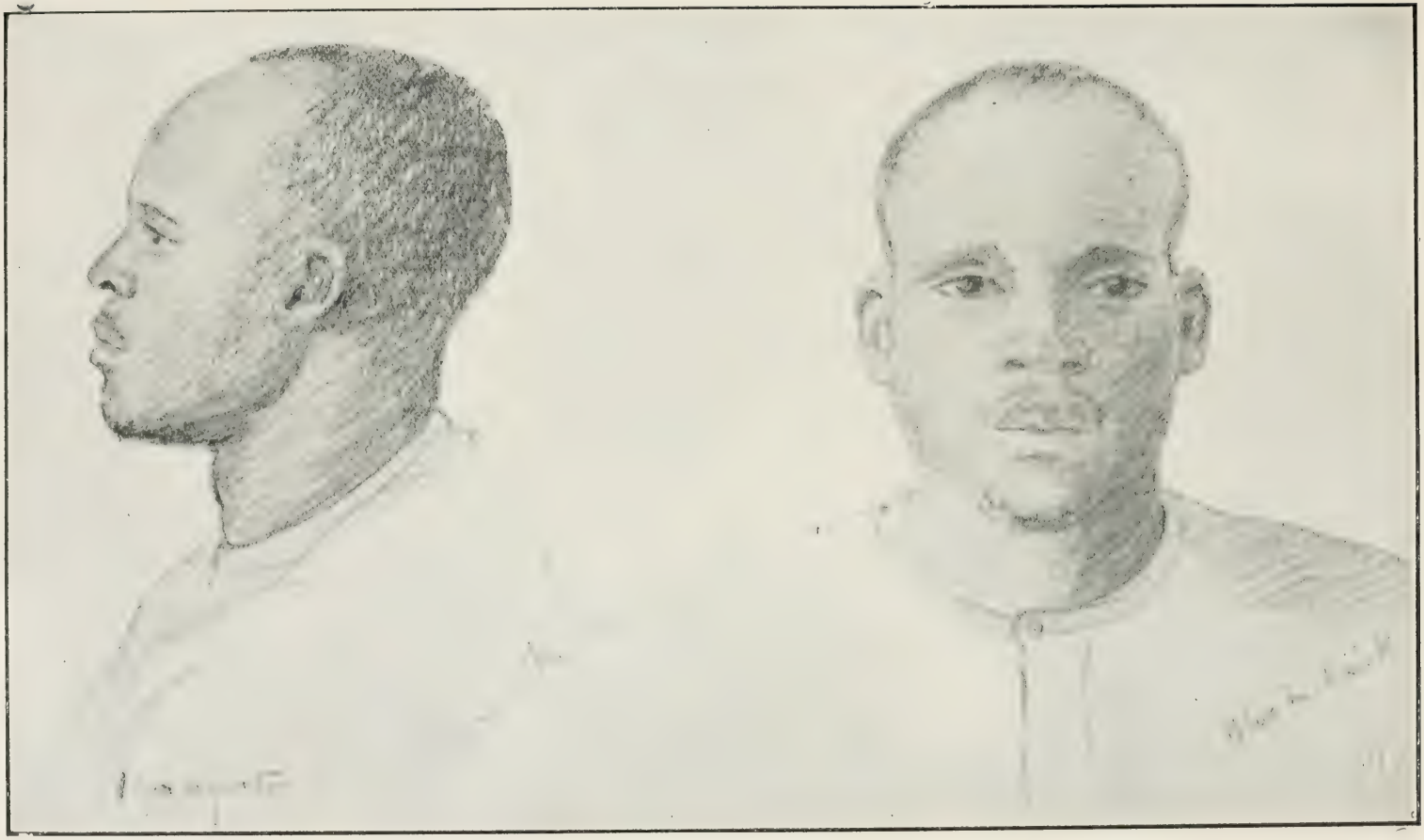
Makua (Arab).



Nyassaland Native.



Yao.



Parapato—Moçambique.

PIANTICATION AND ANAPIANTICATION.

By

A. R. FRIEL, M.D., F.R.C.S.I.

In "The Medical Journal of South Africa" for March and June, 1914, I have recorded the results of some experiments with Friedländer's Pneumo-bacterium and have described a process by which the serological characters of the living organism may be altered The particular process by which the strain of living organism is modified I termed a "piantication"* of the germ.

The aim of the present paper is to describe in greater detail the process of piantication, to emphasise the fact that the change produced in bacteria which have been repeatedly submitted to the action of immune serum is cumulative and transmissible, in a marked degree, to the immediate descendants of the treated bacteria, and to indicate very briefly the practical bearings of the phenomenon.

Special terms are used to denote the changed character of the descendants of the treated bacteria—thus the descendants of those treated by immune serum are called "Pianticated" bacteria, whilst the descendants of those submitted to the action of normal serum are called "Anapianticated" bacteria. The word "Piantication" (or "Anapiantication") is used to denote both the process and its effect, the effect being, as stated above, the production of a strain of bacteria differing in certain definite activities from the original strain.

These newly-acquired characters may be summed up by saying that the pianticated bacteria are rendered more susceptible to the action of normal serum, while the anapianticated bacteria are rendered less so. There is no noticeable change produced in the microscopic appearance of the bacteria or in the character of the growth.

The only organisms which have so far been submitted to the process are the Friedländer Pneumobacterium, a member of the Pasteurella group, and the Pneumococcus. In all of these piantication was successfully achieved.†

An immune serum for the Friedländer bacterium was readily obtained by injecting into the vein of a rabbit a living culture isolated from a case of ozæna; greater difficulty was, however, experienced in preparing an immune serum for the member of the Pasteurella group.

*πιαίνειν = to fatten for slaughter.

†Whilst this paper has been in the hands of the printer the author, with the co-operation of Dr. F. S. Lister, has secured a satisfactory piantication of the Bacillus pestis and a Bacillus coli communis.

as this bacterium when freshly isolated from the nasal cavity of a patient, the subject of ethmoiditis, proved to be very virulent to the rabbit, killing an animal in a few hours when injected into its vein. Success was, however, ultimately obtained by using minimal doses very gradually increased. An immune serum for the Pneumococcus was also obtained by injecting rabbits intravenously with living cultures of a Pneumococcus belonging to Dr. F. S. Lister's variety "C." (*vid.* "Serological Reactions with Pneumococci," S.A.I.M.R., Dec. 22, 1913.) The process took time and one had to proceed with caution to avoid killing the animal.

When the animals had been under treatment for some time their sera acquired the power of agglutinating and opsonising the strain with which they had been injected. In this peculiarity the serum now stood in marked contrast both to the normal serum * of the same animals and also to the author's serum.

The results given in the following table of opsonic observations exemplify this contrast. The numbers refer to the number of leucocytes scrutinised and to the number of bacteria which they were found to contain. In the one case normal serum and in the other immune serum was employed for the opsonic test. The technique employed was that of Sir A. E. Wright.

TABLE I.

	(a) Normal Serum.	*(b) Immune Serum.*
	Friedländer.	
50 Leucocytes ...	1 bact. No agglutination.	53 bact. Marked agglutination.
50 ,, ...	1 bact. do.	80 bact. Agglutination.
	Pasteurella.	
25 Leucocytes ...	8 bact. No agglutination.	Cells crowded. Agglutination.
25 ,, ...	6 bact. do.	61 bact. Agglutination.
	Pneumococcus.	
25 Leucocytes ...	5 bact. No agglutination.	18 bact. Good agglutination.
25 ,, ...	0 bact. do.	37 bact. Good agglutination.

*Experience showed that there was very little difference in results whether normal rabbit serum or normal human serum was used. By "normal serum" in the tables, however, I do not imply the serum of an untreated rabbit but my own serum. This definition applies throughout all the observations here recorded, unless otherwise stated.

Having obtained an immune serum the next step is to treat the bacterium with it; before this is done, however, a certain precaution is necessary.

It is usually conceded that in an immune serum, or in a so-called "normal" serum for that matter, there are two agencies, properties, or bodies, one called "complement" and the other called "immune body." An immune serum differs essentially from normal serum in the fact that the immune body it contains is much more energetic than that of normal serum. The immune body acts on the bacterium and renders it a prey to the complement. The immune body alone cannot cause disintegration of the bacterium, nor can the complement alone, but when the immune body has first acted on the bacterium the process of disintegration or dissolution can then be brought about by adding complement. Immune body and complement can be readily separated in action by the aid of heat, for complement is destroyed by heating to 56° C. for half an hour, while immune body is not. As in these experiments on piantication it is of paramount importance that the bacteria should not be disintegrated or killed it is therefore necessary to destroy the complement by heating the immune serum to 56° for half an hour before allowing it to act on the bacteria.

When the immune serum and bacteria are mixed together agglutination of the bacteria into one or more clumps takes place. After a varying period of contact the agglutinated organisms are removed from the serum, washed in a tube of sterile normal saline solution and then planted on a sloped tube of solid culture media and incubated at 37°. A good growth has usually taken place by the following day and with this new growth the entire process is repeated as often as is necessary to secure a satisfactory degree of piantication. The original untreated culture is subcultured daily to act as a control.

The time during which the bacteria and immune serum are kept in contact has varied in my experiments from 1½ to 24 hours.

The temperature at which the mixture is allowed to stand should be below that at which the bacteria grow readily; "room temperature" is that at which the mixtures have usually been kept in my experiments.

The quantity of serum which must be used is large relatively to the number of bacteria.

The number of occasions on which the organism must be exposed to immune serum before a satisfactory degree of piantication is achieved varies greatly. With Friedländer's bacterium the change has been speedily produced on two or three occasions, but with the Pasteurella and the Pneumococcus numerous exposures were found to be necessary.

Agglutination and opsonic tests, using normal serum, were made with the growth from time to time to detect and measure the changes which the descendants of the treated bacteria were undergoing.

In Table II. is recorded the progress of the piantication of the three strains of organisms. Although the table is an actual record of consecutive observations it should not be supposed that exactly similar results would necessarily be obtained by a repetition, nor that the process of piantication would necessarily be as readily carried out. The table does not record, for instance, fourteen exposures of the Pneumococcus to immune serum (for about four hours daily) between November 24th and December 9th. No piantication effects followed any of these exposures, a fact which was thought to be due to feebleness of the immune serum used.

TABLE II.

SHOWING PROGRESS OF PIANTICATION.

	Treated Strain.			Untreated Strain. (Subcultured daily).	
Date of Treatment, Test, and Subculture.	Duration of Contact with Immune Serum.	Opsonic Test with Normal Serum.		Opsonic Test with Normal Serum.	
		Bacteria.	Cells.	Bacteria.	Cells.
*Friedländer's Pneumobacterium. (From a case of Ozæna.)**					
March 27 ...	4 hours.			17 in	50
28 ...	5 ,,	9 in	30		
30 ...	5 ,,	62 ,,	50	6 ,,	50
Pasteurella. (From a case of Ethmoiditis.)					
Dec. 22 ...	5 hours.	9 in	50	11 in	50
23 ...	12 ,,	3 ,,	25	3 ,,	25
24 ...	12 ,,	4 ,,	25	5 ,,	25
28 ...		1 ,,	25	3 ,,	25
29 ...		1 ,,	25	1 ,,	25
30 ...		4 ,,	25	5 ,,	25
31 ...		3 ,,	25	8 ,,	25
Jan. 2 ...		14 ,,	25	5 ,,	25
4 ...	4 ,,	7 ,,	25	5 ,,	25
5 ...	2 ,,	9 ,,	25	8 ,,	25
8 ...	3 ,,	5 ,,	25	1 ,,	25
9 ...	24 ,,	3 ,,	25	4 ,,	25
11 ...		7 ,,	25	1 ,,	25
12 ...		6 ,,	25	1 ,,	25
13 ...		4 ,,	25	3 ,,	25
14 ...	5 ,,	33 ,,	25	3 ,,	25
15 ...	6 ,,	84 ,,	10	5 ,,	25
16 ...	6 ,,	122 ,,	25	5 ,,	25

Pasteurella—continued.

Date of Treatment, Test, and Subculture.	Duration of Contact with Immune Serum.	Treated Strain. Opsonic Test with Normal Serum. Bacteria.	Cells.	Untreated Strain. (Subcultured daily) Opsonic Test with Normal Serum. Bacteria.	Cells.
Jan. 18 ...	24 hours.	43 in	5	1 in	25
19 ...	5 ,,	109 ,,	25	2 ,,	25
20 ...	5 ,,	83 ,,	25	1 ,,	25
21 ...	4 ,,	142 ,,	25	6 ,,	25
22 ...	4 ,,	96 ,,	50	5 ,,	25
23 ...	4 ,,	55 ,,	10	3 ,,	25
25 ...	24 ,,	42 ,,	10	3 ,,	25
26 ...	7½ ,,	33 ,,	5	4 ,,	25
27 ...	7 ,,	35 ,,	5	3 ,,	25

Pneumococcus. (Dr. F. S. Lister's Variety "C.")

			Cocci.		Cells.	Cocci.		Cells.
Dec. 9 ...	3–5 hours.	2	in	25	0	in	25
10 ...	,, ,, ,,	15	,,	25	5	,,	25
11 ...	,, ,, ,,	12	,,	25			
12 ...	,, ,, ,,	11	,,	25	1	,,	25
13 ...	24 ,,						
14 ...	5 ,,	58	,,	25	0	,,	25
15 ...		17	,,	25			
16 ...	24 ,,						
17 ...		33	,,	25	5	,,	25
18 ...	24 ,,						
19 ...		15	,,	25	0	,,	25
20 ...	24 ,,						
21 ...	24 ,,	35	,,	25	4	,,	25
22 ...	12 hours.	35	,,	25	2	,,	25
23 ...	24 ,,	27	,,	10			
24 ...		68	,,	25			
27 ...	24 ,,						
Dec. 28 ...	12 ,,	103	in	25	1	in	25
29 ...	10 ,,	72	,,	25	3	,,	25
30 ...	10 ,,	60	,,	25	1	,,	25
31 ...		42	,,	5			
Jan. 2 ...		187	,,	25	6	,,	25

*This organism was the first to be submitted to investigation and the desirability of keeping fuller records of observations was not appreciated at the time.

It is to be noted that the opsonic tests recorded in this table were made with *normal* serum only. The organisms which were previously almost entirely resistant to phagocytosis, using normal serum (cf. also Table I.), were finally readily phagocyted. It is also to be noted that the normal serum is occasionally able to agglutinate the culture. These facts indicated that the strain of organism had now definitely changed its serological characters, and this change may be briefly described by saying that the organism had become "pianticated."

It seems probable that the changes due to piantication are transmitted through, at all events, two subcultures of the micro-organism. The following observations showed that the first subculture from the pianticated Pasteurella was as active as its immediate parent and that the piantication characters were still present, in a high degree, in the second subculture.

	Leucocytes.	Bacteria.
Pianticated Pasteurella	5	37
1st Subculture. (24 hours) ...	5	40
2nd ,, ...	5	28
3rd ,, ...	25	11
4th ,, ...	25	4

If opsonic tests such as are recorded in Table II., and agglutination tests, be made, substituting *immune* serum for *normal* serum, another striking phenomenon becomes apparent, namely, the immune serum has now, as a rule, less effect on the pianticated strain than on the original strain. It is frequently found that the immune serum does not agglutinate the pianticated strain, or only slightly, and further, that it may not opsonise it so well as it does the original strain. The following results were obtained with Friedländer's bacterium and *immune* serum:—

	Original Friedländer.	Pianticated Friedländer.
25 Leucocytes.	80 Bacilli.	26 Bacilli.

The practical application of the process of piantication is obviously in the domain of vaccine therapy. The bacterial vaccines now most generally used consist of killed organisms, and the potency of the vaccine is regulated solely by varying the number of bacterial bodies injected. Vaccination with living organisms probably produces a more lasting immunity than when killed organisms are used, and the fundamental objection to their use has been to a large extent overcome by Prof. Besredka's discovery of sensitised vaccines. The essential feature of Professor Besredka's discovery is the reduction of the virulence of the organism owing to the treatment it has received. Piantication is an extension of our knowledge in this particular direction.

Piantication may be provisionally described as a sensitisation accumulating through many generations of the culture. It enables us to produce vaccines which are graduated as regards their virulence.

The quantity of any particular Besredka vaccine which can be produced depends mainly upon the quantity of immune serum which is available. When, however, a strain of organism has been pianticated the amount of vaccine which may be obtained from it is limited

only by our facilities for cultivating the organism in bulk. About one-third of an agar slope of the pianticated Pasteurella was emulsified in saline and used to inoculate ten large agar bottle cultures. The amount of culture obtained in twenty-four hours was estimated to be three hundred times the original. The growth in each of the bottles was submitted to opsonic test and in all cases was found to be even more actively pianticated than the original.

It is probable that the effects following the injection of living pianticated organisms are of the same nature as those following the use of living sensitised vaccines. Mice which have received subcutaneous doses of pianticated Pneumococci have survived whilst similar doses of the same organism in an unpianticated state have proved fatal to the control animals. That the pianticated injections are antigenic and lead to the development of antibacterial properties is shown by the following table of observations:—

TABLE III.

Rabbit (1·162 kilos) treated intravenously with living, pianticated Friedländer.

Date.	Injections.	Opsonic Tests.
April 10 ...		0 bact. in 25 cells.
,, 14 ...	12½ million.	
,, 20 ...		4 ,, ,, ,, ,,
,, 20 ...	20 million.	
,, 24 ...		11 ,, ,, ,, ,,
,, 29 ...		67 ,, ,, ,, ,,
		Slight agglutination.

In addition to these possible practical applications the process of piantication is of some academic interest in that it affords a means of temporarily changing the characteristics of at least some bacteria in a certain direction, namely, their resistance to phagocytosis and agglutination, and this is accomplished without interference with their vegetative powers.

To effect piantication a very energetically immune serum must be employed; a weak serum is useless, in fact prejudicial to the object in view. This peculiarity invites a parallel which is suggestive:— There is a general law regarding the action of narcotic drugs that a small dose stimulates whilst a large dose paralyses the nerve cells. If an immune serum be considered to act as a "paralyser" of the bacterium a normal serum might, by analogy, be considered to act as a stimulant. All stimuli are irritants, or in other words immediately injurious, in that they lead to the disintegration of protoplasm. Although the immediate effects of a stimulus may be injurious the remote effect may be beneficial, for, in accordance with Weigert's law of "Over production as a result of Injury," the products of re-action

in response to a moderate stimulus are greater than the immediate destruction. This principle may possibly be applied to explain why a bacterium is increased in virulence by inoculating it into a susceptible animal; the feebly immune property of the animal's serum acts as a stimulant to the virulence of the organism. The experiment to be now detailed seems to support such an explanation.

It is a well-known fact that by repeated sub-cultivation on artificial media a pathogenic organism usually loses its virulence, and that an organism which originally resisted phagocytosis may become readily phagocytable. The Pasteurella and Friedländer organisms already referred to, after repeated subcultivation, became opsonisable by normal serum. Normal serum, in the light of what has been said above, may be regarded as a weak stimulus to the bacterium. An emulsion of the phagocytable Pasteurella was therefore mixed with normal serum, left in contact for three or four hours, and then planted; on the following day the growth was tested and these results were obtained:—

	Leucocytes.	Original Strain.	Anapianticated Strain.
Tested with Author's own serum	25	19 Bacteria.	0 Bacteria.
	,,	45 ,,	2 ,,
Tested with serum of rabbits in process of immunisation* ...	,,	58 ,,	19 ,,
	,,	49 ,,	0 ,,
	,,	49 ,,	3 ,,
	,,	60 ,,	8 ,,

*It was definitely ascertained that these sera had not developed immune properties to any extent.

These results seem to show that exposure to normal serum restores to an organism the power to resist phagocytosis when this has become lost by prolonged subcultivation.

If the process by which a resistant strain, when treated with immune serum, is rendered more phagocytable be called "piantication," that of treating a bacterium which is losing its power of resisting phagocytosis owing to repeated subcultivation, and rendering it again less phagocytable, may be called "anapiantication."

In conclusion, it is apparent that we are now in possession of a means of controlling the virulence of certain pathogenic bacteria. It is possible that a large number of the pathogenic micro-organisms will be found to be amenable to the process. The observation may open up new fields in vaccine treatment, both preventive and curative, and also appears to be of academic interest.

Miss Michaelis has assisted me in the laboratory and I also wish to acknowledge the help which I have received from the criticisms of Drs. M. Blieden and F. S. Lister, and the assistance of the Director of the Institute, Dr. W. Watkins-Pitchford.

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THE TRYPANOSOMES OF SLEEPING SICKNESS; BEING A STUDY OF THE GROUNDS FOR THE ALLEGED IDENTITY OF *T. BRUCEI* WITH THOSE CAUSING DISEASE IN MAN IN NYASALAND.

By G. D. MAYNARD, F.R.C.S., E.

The recent report, No. XVI., of the Sleeping Sickness Commission of the Royal Society is of great interest and importance to all African Governments, and neither the conclusions arrived at nor the recommendations made can be lightly ignored by those interested in the welfare of this Continent. In order to indicate the nature of the recommendations a few extracts will be quoted. It will be noted that the proposals made are of such importance to the inhabitants of this country that great caution should be observed before they are seriously entertained, and that the evidence on which they are based calls for most careful scrutiny. It is no doubt comparatively simple to propose remedies which entail the adoption of measures of far-reaching economic and political importance, but the inhabitants of the country who will inevitably reap the aftermath, if such proposals are put into practice, will doubtless demand some unanimity of expert opinion, and a reasonable probability of success, before they decide to carry out such recommendations.

The Commission, having satisfied itself that the Sleeping Sickness of Nyasaland and Rhodesia is caused by a trypanosome which, it states, is common in all "fly-country" and is spread from wild animals to man, recommends "that efforts should be made to diminish, as far as possible, the number of wild animals in fly-areas. Every wild animal destroyed or driven out is one less source or centre of infection." . . . "As to the means of bringing about this diminution of wild game in fly-areas, the Commission is aware of the great difficulties lying in the way. It is not practicable to arm the natives to any extent with arms of precision. . . . The natives should be allowed to pursue any of the ordinary wild animals without let or hindrance. They might use their own methods, such as game-pits or traps, and they should be permitted to use a certain number of useful guns." (Report, p. 6.)

Let us consider for a moment the effect of this recommendation. If the Commission is in error in supposing all game in *Glossina morsitans* areas to be a danger to human beings, and if it is only in certain districts that the game are the carriers of a trypanosome harmful to man, then the effect of this proposal would probably be to drive the dangerously infected game into zones which are at present uninfected. As that well-known sportsman, D. D. Lyall, writes—in "Wild Life in Central Africa"—"The extermination of game in a short space of time is absolutely impossible in such countries as Nyasaland and Northern Rhodesia, for these are heavily bushed and grassed regions, and game, when much molested, simply moves on until it discovers a locality where it is safe from molestation." For this recommendation to be valid two suppositions must be confirmed, namely:—(*a*) that wild game in all districts where *G. morsitans* is found are carriers of the trypanosome causing human trypanosomiasis, and (*b*) that if the game is eliminated the smaller animals will not act as carriers. Clearly if these premises are not correct the proposal may do much harm, and its adoption may even assist in spreading the disease.

It is proposed, then, that attempts should be made to destroy all game in fly-areas, whether such areas are or are not known to be Sleeping Sickness districts. A glance at a map, such as that in E. E. Austin's "Handbook of the Tsetse-Flies," will show what this proposal involves, namely, the denudation of the Continent of game over an extensive area south of latitude 15° N.; that is, from the Senegal River on the west, through Lake Chad and north of the Lado, to the northern border of British East Africa, and on the south from the Cunene River on the west, through Lake Ngami, along the Limpopo, Eastern Transvaal, to Zululand on the east. Such then, roughly, is the area affected by this proposal. The enormous economic importance of such a recommendation will be obvious to all who know anything of the life and habits of the millions of natives inhabiting these districts.

The reader who has not carefully studied the Commission's report may perhaps think too literal an interpretation is being placed on the phrase "in fly-areas," and that this was intended to apply only to known Sleeping Sickness districts. The following extracts will perhaps make the view of the Commission clearer. "In the opinion of the Commission it is probable that cases" (*i.e.*, of Sleeping Sickness) "will be found to occur wherever *Glossina morsitans* and wild game abound." . . . . "This parasite" (*i.e.*, the trypanosome they claim to be a cause of Sleeping Sickness) "is found in the blood of wild animals throughout a large part of Africa" (p. 30.) It is also of the opinion

that cases of Sleeping Sickness must have occurred in Zululand, although they have not been discovered. In reference to this latter district, it remarks:—" Many cases of death are reported to have occurred among hunters and explorers, and these were often put down to ' Malaria '; but as there was no blood examination, it is quite possible that some of these cases might have been due to infection by *Trypanosoma brucei*. It is doubtful if man ever dies of uncomplicated malaria " (p. 35). In the first sentence of this extract, we have what an unkind critic might term a *suggestio falsi*, in that while the statement—that many men have died without a blood examination having been made—is true, it is not the whole truth; for, to suggest—and without such suggestion the sentence has little real significance—that men in recent years dying of obscure diseases in Zululand or the Eastern Transvaal have not in many instances had blood-examinations made, is certainly quite incorrect. When we consider that Sleeping Sickness was discovered, whilst still infrequent, in districts much more isolated and much further removed from laboratory aid than Zululand, it does not seem at all probable that it has been consistently missed in a district which is, medically speaking, relatively well supervised. The final statement of the extract is even harder to understand, unless possibly the word " uncomplicated " is to nullify the surface meaning of the remark. If this sentence does not mean that for all practical purposes Malaria as a cause of death can be ignored its introduction here is certainly likely to mislead readers unfamiliar with tropical diseases. Medical practitioners in the tropics, knowing that such a statement is incorrect, will no doubt wonder what the Authors intended to convey. For the reader unacquainted with tropical diseases the following quotations may be of interest as indicating the importance of Malaria as a cause of death. " The true mortality of Malaria is difficult to estimate. . . . It is probably true that many cases ascribed to complications, fancied or real, are due to Malaria." " The following list of pernicious Malaria compiled from literature shows a mortality of 26·6 per cent." (Deaderick, " Practical Studies of Malaria," 1909, p p. 296-297.) " . . . something will be done to lessen the terrible mortality from Malaria among native children in India." (Rogers, " Fevers in the Tropics," p. 201.) " There can, however, be no doubt that Malaria causes the greatest amount of sickness in all tropical countries and may in certain localities be largely responsible for the death-rate, while in others it may not contribute nearly so largely." (Castellani & Chalmers, " Tropical Medicine," 1910, p. 672.)

It is evident that when the expression " destruction of wild game in fly-country " is used, it is not intended to limit the destruction to districts where Sleeping Sickness is known to exist. The far-reaching

effect of this recommendation is plain, and very valid arguments in support of the contentions on which it is based must be adduced before African Governments should attempt to follow such advice.

Among other preventive methods suggested is the segregation of infected natives, but after stating "Therefore it would be well to move infected natives out of the fly-country into fly-free parts," the proposal is dismissed with "This, however, is not an important matter." Moreover, if the conclusions of the Commission are sound —that all tsetse fly areas are Sleeping Sickness districts—the proposal is impracticable; if, on the other hand, the assumptions made in regard to wild game are incorrect and the native is the most important source of infection, the suggestion in spite of administrative difficulties deserves more serious consideration, and cannot be dismissed as an unimportant matter.

It is unnecessary to labour the gravity of the Commission's recommendations and statements provided the evidence on which they are based is established and the deductions are sound. If Sleeping Sickness, as they claim, exists in the Union of South Africa but is unrecognised, it is a serious matter and deserves the most careful investigation and consideration. The opinion that Sleeping Sickness occurs in hitherto unsuspected areas is apparently entirely based on the presumed identity of the *Trypanosoma brucei* with that causing Sleeping Sickness in man in Nyasaland. It is justifiable, therefore, to examine somewhat carefully the evidence on which this presumed identity is based.

The Commission states (p. 35) that seven points may be considered in the endeavour to separate species of Trypanosomes:—

"1. Are they alike in size and shape? Morphology.
"2. Have they the same habits and actions when alive?
"3. Do the two trypanosomes infect the same animals and kill them in the same time?
"4. Have they the same carrier? and does their growth or development in the carrier follow the same course?
"5. Cross-inoculation experiments.—If an animal has recovered from an attack of disease set up by one of the two trypanosomes, is it immune or non-immune to infection by the other?
"6. Experiments on the effect of various sera.
"7. Geographical distribution."

Before considering these points in detail it may be remarked that the Commission considers the matter to be definitely settled, for in the definition of terms at the beginning of the report *T. brucei* is defined as: "The parasite found in wild game and tsetse flies of

Nyasaland and many other parts of Africa. It gives rise in man to the ' Nyasaland Sleeping Sickness,' and in other animals to the disease known as ' Nagana.' In this Report it will be designated *T. brucei vel rhodesiense.*" The trypanosome found in wild game and tsetse flies of Nyasaland is thus labelled, without any qualification, as being the *Trypanosoma brucei*, and as being identical with the organism causing disease in man. Similar statements are repeated so often that all but the most careful readers may consider, by the time that they have read through the report, that the matter is definitely settled, and such isolated remarks as the following may be overlooked or forgotten:— "This (the question of the identity of *T. brucei* and *T. rhodesiense*) is a most important question, and, with our present knowledge, *one impossible to answer at the present time with absolute certainty.*" (p. 33, italics not in the original.) "These 25 strains, or races, are all believed by the Commission to belong to the same species —*Trypanosoma brucei.* This belief is not shared by the majority of workers on trypanosome diseases. It is held that mixed up with *T. brucei* there is another species, *T. rhodesiense*, which is alone capable of giving rise to disease in man." (p. 42.) Or again (p. 36)— "It will be found difficult, or even impossible, to arrive at a certain decision in regard to the identity of *Trypanosoma brucei* and *Trypanosoma rhodesiense.* Many apparently cogent arguments can be advanced against this theory." With the exception of the above extracts there is little indication to show that the Commission considers that any doubt remains as to the identity of these species.

There are, therefore, two main questions to be investigated—Is the wild game strain of Nyasaland identical with the strain causing disease in man? and, if so, is this trypanosome identical with the *T. brucei* of Zululand? Should it be found that there is more than one wild game strain in Nyasaland, or more than one strain causing disease in man, a further complication will result.

1. *Morphology.*

In regard to this, the Authors of the report state that it is "a most important point." The morphological feature which they apparently consider of greatest moment is length, and the numerous graphs of frequency-distributions of length-measurements indicate the value they place on this method of examination for purposes of classification.

Many other writers on trypanosomes have attached similar importance to this morphological feature. Warrington Yorke and B. Blacklock in an interesting paper, entitled "The differentiation of the more important Mammalian Trypanosomes" (Annals of Trop. Med.

and Parasitology, April, 1914) discuss the value of morphological features for purposes of differentiation. In referring to the graphs of the Royal Society Commission they remark—"On comparing these graphs, one is at once impressed by the extraordinary variation they exhibit." They then discuss the probable reason for these variations, and write—"In conclusion, therefore, we are of opinion that in so far as polymorphic trypanosomes are concerned the character of biometric curves affords no assistance in diagnosis." In regard to the unanalysed graphs this conclusion is no doubt just as applied to bi-modal or multi-modal distribution, and one is almost inclined to agree with their statement—"If they are at all similar, it would appear to be chiefly a matter of coincidence." Unfortunately the antecedent treatment of these strains (such as animal-passages) was not sufficiently uniform to enable a definite and final opinion on this point to be formed. Whether the ratio of the components would be found to be more or less uniform from strain to strain in the same species of trypanosome, if the animal passages and so forth were identical, is, I think, impossible to assert on the evidence available; there are, however, certain facts which suggest that probably this would not be so, and that the statement above quoted is, therefore, correct. The Authors of this paper have discussed this point entirely from an inspectional standpoint and appear to be unacquainted with the methods of the scientific analysis of such distributions, or of the statistical laws on which alone can sound deductions as to similarity or dissimilarity be based. Their criticism is of interest, however, as showing how greatly their conclusions differ from those of the Commission even when based on the same facts and judged by the same methods, namely, mere inspection of the graphs.

As pointed out by Professor K. Pearson, in an article on "The Identity of Trypanosome Strains" (Biometrika, Vol. X., p. 85 *et seq.*)—"It is impossible to reach any safe conclusions as to the identity or non-identity of populations by any process of mere graphical comparison of frequency distributions." In this paper the chance that so-called similar distributions are really samples from a *homogeneous* population is discussed, and it is shown that, considering the distributions as they stand, there are in many cases chances of millions to one against similarity where such is claimed by Sir David Bruce and his colleagues. This paper was published about a year before the report now under consideration was issued, but no mention is made of these criticisms and the same inspectional methods of judging similarity is still adhered to.

It is very difficult to discover what, in the opinion of the Authors of the report, constitutes morphological similarity. For example, on

p. 87 it is stated—"As regards morphology, this trypanosome (*T. brucei*) is absolutely identical with the Trypanosome causing disease in man in Nyasaland, the *T. rhodesiense* of Stephens and Fantham." Only a few lines above, however, the following remarks are made:—"These two curves are undoubtedly much alike, and, as the wild-game strain in Nyasaland is supposed to be identical with the human strain, then it might be said that *T. brucei*, Zululand Strain XXV., 1913, is also identical with the trypanosome causing disease in man in Nyasaland." In regard to the undoubted similarity of the two strains the chances that the variations observed in the charts are due simply to errors of sampling are millions to one against; these appear to me, therefore, rather slender data on which to assert their absolute identity. Further, the *T. brucei* Strain XXV. was, it is stated, "in all probability infected from the wild game strain of the Somkele District, Zululand": thus even the origin of this strain is not definitely ascertained.

Just as it is possible to pick out two frequency-distributions which may, to the superficial observer, appear similar, so from among the same strains can distributions be picked out which no reasonable observer could, in their crude state, consider as even approximately representing similar length-measurement-distributions. Chart I. gives four histograms of length-measurements taken from the report. All these measurements are from strains the identity of which is asserted by the Commission. Whereas (*a*) and (*b*) are somewhat similar in type their position is different, the commonest modal and approximately mean length in the (*a*) distribution occurring at about 19 microns and in (*b*) at 24; (*c*) and (*d*) are different in type to both the foregoing and to each other. It will further be noted that both (*a*) and (*d*) although so dissimilar are strains of *T. brucei*. Chart II. shows a strain of *T. gambiense* and one of *T. rhodesiense*. Although the Commission regard these trypanosomes as distinct species, the histograms show greater similarity than those given in Chart I. It is not, of course, claimed that these distributions do in fact show similarity, they are introduced here merely as a contrast to those in Chart I. Even greater similarity is observed between the distributions of *T. gambiense* and *T. rhodesiense* given by Stephens and Fantham and reproduced in Charts III. and IV., but those in Chart II. were chosen because the diagrams were given in the Commission's report. The Commission states:—"It is doubtful if it is possible in every case to distinguish *T. brucei* from *T. gambiense* by morphology alone." (p. 30.) This would suggest that in some cases it was possible to distinguish the two by their morphology. However, we are told on p. 196 that "It would appear to be impossible at present to

distinguish between the two by microscopic examination of the parasites as they occur in the blood of man." So that presumably, where it is possible to distinguish the strains by their morphology, it is to length-measurements that appeal must be made. If, however, these two species, claimed as distinct, cannot be distinguished by any morphological feature there can be no justification for claiming similarity for *T. brucei* and the Nyasaland human strain, from assumed similarity in distributions of length-measurements.

From the consideration of the above mentioned facts, and the reproduced charts, the unbiased reader will agree with the conclusion that little information of value can be obtained from the mere inspection of diagrams of frequency-distributions of trypanosome lengths, and that any arguments drawn from such sources, whether in support of the similarity or dissimilarity of species, can be of but little worth. Are we then to abandon all this information as valueless? Whatever one may feel about many of the deductions drawn by the Commission in this matter, one cannot but be grateful to them for the labour expended on these measurements, and every attempt should be made to extract what information is possible from such work.

It is asserted that both *T. brucei* and *T. gambiense* are dimorphic strains. In the paper by Professor Pearson, already referred to, he has shown that length-measurement-distributions of *T. pecorum*, *T. simiæ*, *T. capræ* are well represented by a single "normal curve." On the other hand, the distributions of *T. gambiense*, *T. rhodesiense*, wild game strain, *T. brucei*, etc., cannot, at least in many instances, be reasonably represented by single "normal curves." Further, he has shown that on analysis some of the above-mentioned strains split into two components, each of which is well represented by a "normal curve." Without discussing, in this place, the significance of this phenomenon the fact may be noted. It is proposed for the present to continue to call such strains "dimorphic," and the question as to whether the compound nature of these distributions be due to a mixture of two strains or a true dimorphism is left in abeyance. If in addition to the presence of two components in these distributions we assume that their relative proportion may vary, it will be evident that great differences in the distributions of measurements of length may occur even when the same strain is being dealt with. A simple example may help to make this important point clear.

It is well known that measurements of heights in both men and women are well represented by the "normal curve," and, therefore, give distributions similar in character to those just discussed in regard

to the trypanosome strains. Let us assume then, that we are trying to distinguish, say, a European from the Southern Chinese race by height-measurements. In each case the mean height of the two sexes differs considerably. It is not, of course, to be inferred that because an illustration from sex differences is being made use of any suggestion that a sex difference is important in the trypanosome strains is implied. To return—Suppose 1,000 measurements of height were made at random among the sexes the resulting type of distribution would depend, to a large extent, on the proportion of males to females in the group. If in the European sample the female type largely predominated, and in the Chinese the male, the two distributions might appear somewhat similar if mere inspection were relied on, and consequently sameness of race might be claimed. In the same way, in two different samples from the same race, difference of race might be alleged if considerable differences in the proportion of males to females were present in the samples.

This illustration will, I hope, help to demonstrate the impossibility of judging similarity or dissimilarity of such compound curves before they have been resolved into their components. The extract quoted from Professor Karl Pearson gives his opinion as to even simple distributions, and I am certain all trained statisticians will endorse this view; but when compound distributions are under consideration even the "goodness of fit" tests fail to answer the question of similarity of origin until an analysis into components has been made, for the obvious reason that the population is not really homogeneous in respect to the character measured.

Professor Pearson has shown that many of the so-called "dimorphic" trypanosome strains can be resolved into two "normal-curve" components, and the theoretical distribution which results from the sum of the areas of these curves can then be compared with the original distribution and the "goodness of fit" measured by the well-known formula.

Having had some difficulty in resolving certain distributions of human strains given in the Commission's report, and noticing that Professor Pearson's analysis of human strains I. to V. did not result in a reasonable fit, it seemed advisable to test another sample of both *T. rhodesiense* and *T. gambiense* with a view to ascertaining whether the good results obtained by Professor Pearson with these strains would be repeated. A distribution of 1,000 counts of each of these strains was obtained from Stephens and Fantham's paper ("Annals of Tropical Medicine and Parasitology," Vol. VII., pp. 28 and 32), and their analysis leads to the following results:—

T. rhodesiense.

Mean = 24·1210

$\mu_2 = 12{\cdot}3386$ $\quad$ $\mu_3 = -\ 5{\cdot}5178$

$\mu_4 = 396{\cdot}2356$ $\quad$ $\mu_5 = -335{\cdot}8823$

Reducing nonic:—

$$q^9 - 2{\cdot}1170q^7 + {\cdot}04567q^6 + {\cdot}8010q^5 - 1{\cdot}2327q^4 - {\cdot}7559q^3 - {\cdot}01184q^2 + {\cdot}002243q - {\cdot}000{,}028{,}22 = 0.$$

The root is given by $p_2 = 10q = -5{\cdot}2889$, which leads to the components given in Table I. and shown in Chart III. $P = {\cdot}54$.

T. gambiense.

Mean = 24·867

$\mu_2 = 12{\cdot}0113$ $\quad$ $\mu_3 = -\ 4{\cdot}2121$

$\mu_4 = 366{\cdot}7023$ $\quad$ $\mu_5 = -234{\cdot}2183$

Reducing nonic:—

$$q^9 - 2{\cdot}3139q^7 + {\cdot}02661q^6 + 1{\cdot}2957q^5 - {\cdot}7707q^4 - {\cdot}6618q^3 - {\cdot}01426q^2 + {\cdot}008324q - {\cdot}000{,}055{,}84 = 0.$$

The root is given by $p_2 = 10q = -5{\cdot}2641$, which leads to the components given in Table I. and shown in Chart IV. $P = {\cdot}48$.

The close similarity of type of these two distributions and their constants will be noted.

Turning to the Nyasaland human strains. In this group five strains (Human Strains, Nyasaland VI.—X.) are represented, all of which were taken from the rat. (p. 57.)

Mean = 22·2872

$\mu_2 = 12{\cdot}0126$ $\quad$ $\mu_3 = 20{\cdot}0529$

$\mu_4 = 392{\cdot}0418$ $\quad$ $\mu_5 = 1664{\cdot}7845$

Reducing nonic:—

$$q^9 - 1{\cdot}4303q^7 + {\cdot}6032q^6 - 3{\cdot}8501q^5 - 7{\cdot}1924q^4 - {\cdot}8802q^3 + 1{\cdot}6237q^2 + {\cdot}2643q - {\cdot}06502 = 0,$$

which leads to $p_2 = 10q = -5{\cdot}3916$. The components are given in Table I. and shown in Chart V. $P = {\cdot}77$.

Human Strains—Nyasaland I.—V.

This group has been dealt with by Professor Pearson. The areas of his components, however, do not well represent the data, and only about once in 6,000 samples should so unsatisfactory a fit be obtained if the divergence be due to errors of sampling. Professor Pearson

writes:—" It is impossible, however, to look through the graphs given by Sir David Bruce and others for the human strains without being convinced of their fundamental bi-modal character, although there appears to be much evidence of its being disguised by heterogeneity of host and treatment." On examining the graphs of these five strains it appeared to me that Strain III., the Chituluka Strain, was possibly different in character to the others. Professor Pearson had already suggested that as it was not at all satisfactorily fitted by two components a third might be present. I, therefore, took the remaining four strains (through rat only) and attempted to fit them with two components. This distribution gave the following constants:—

Mean = 23·9363

$\mu_2 = 10{\cdot}3011$ $\mu_3 = 13{\cdot}5416$

$\mu_4 = 293{\cdot}6205$ $\mu_5 = 1025{\cdot}3776$

Reducing nonic:—

$$q^9 - {\cdot}86520q^7 + {\cdot}25512q^6 - 1{\cdot}01528q^5 - 1{\cdot}53628q^4 - {\cdot}13098q^3 + {\cdot}18437q^2 - {\cdot}02860q - {\cdot}004{,}920{,}2 = 0.$$

No suitable root to this equation could be found.

As the resolution of the group of human strains I. to V. was unsatisfactory it was decided to deal with each strain separately to test how far the mixture of strains affected the results. To eliminate as far as possible heterogeneity, due possibly to alterations in character produced by different immediate hosts, only measurements obtained from rats were used.

Human Strain I.

Mean = 25·1367

$\mu_2 = 17{\cdot}5847$ $\mu_3 = -\ 0{\cdot}6998$

$\mu_4 = 602{\cdot}4457$ $\mu_5 = 393{\cdot}7376$

Leading to reducing nonic:—

$$q^9 - 11{\cdot}3805q^7 + {\cdot}00073q^6 + 39{\cdot}5390q^5 - 2{\cdot}0325q^4 - 39{\cdot}2039q^3 - {\cdot}01354q^2 + {\cdot}000{,}003{,}12q - {\cdot}000{,}000{,}000{,}1 = 0,$$

which gave $p_2 = 10q = -12{\cdot}1344$, which led to the components given in Table I. and shown in Chart VI. $P = {\cdot}62$. So that the resolution of this strain into two normal components was quite satisfactory.

Human Strain II.

Mean = 23·160

$\mu_2 = 6{\cdot}6271$ $\mu_3 = 4{\cdot}7349$

$\mu_4 = 134{\cdot}1327$ $\mu_5 = 306{\cdot}3063$

Which led to nonic:—

$$q^9 + \cdot08325q^7 + \cdot03363q^6 - \cdot008503q^5 + \cdot009446q^4 + \cdot006396q^3 + \cdot0002049q^2 - \cdot000,047,82q - \cdot000,011,27 = 0.$$

No negative real root could be found for this equation; but the 1,000 measurements which formed this distribution were derived from two rats, and Professor Pearson had pointed out that these two distributions did not resemble each other, for $\chi^2 = 36\cdot195$ and $P = \cdot0048$. To what, therefore, was this want of agreement attributable? If the two sub-distributions differed only in the fact that the areas of their components (presuming they were compound distributions) were different, the low value for P quoted above, might be explained, although this would not affect the resolution of the combined distribution; if, however, the components were of a different character the heterogeneity would affect the resolution.

The two sub-populations were then dealt with separately.

Human Strain II. A., Rat 728.

Mean = 23·516

$\mu_2 = 6\cdot2504$ $\quad\quad$ $\mu_3 = 5\cdot0100$

$\mu_4 = 114\cdot3804$ $\quad\quad$ $\mu_5 = 223\cdot5815$

Leading to reducing nonic:—

$$q^9 - \cdot09879q^7 + \cdot03765q^6 - \cdot13162q^5 - \cdot07327q^4 + \cdot001836q^3 + \cdot003326q^2 + \cdot000,071,12q - \cdot000,015,81 = 0.$$

The root was found to be $p_2 = 10q = -2\cdot4884$, leading to the components as given in Table I. and shown in Chart VII. In this case $P = \cdot88$, a quite satisfactory result.

Human Strain II. B., Rat 726.

Mean = 22·804

$\mu_2 = 6\cdot7503$ $\quad\quad$ $\mu_3 = 4\cdot9937$

$\mu_4 = 143\cdot9428$ $\quad\quad$ $\mu_5 = 429\cdot6443$

Reducing nonic:—

$$q^9 + \cdot25358q^7 + \cdot03741q^6 - \cdot15835q^5 - \cdot03083q^4 - \cdot007179q^3 - \cdot007,179q^2 - \cdot000,180,2q - \cdot000,015,5 = 0.$$

This equation has no real negative root, and therefore the distribution cannot be strictly resolved into two components, nor will a single normal curve satisfactorily represent the observations, as will be seen on reference to Chart VIII.

The difference in these two examples of Strain II. is curious, and some biological explanation probably exists. The histogram rather suggests that a third component may be present in the distribution

obtained from rat 726. Such anomalies as are found in this, and some other strains to be discussed later, may hide important biological facts, but as long as workers on this subject are content with mere inspection of graphs, or a rough analysis into three groups —short, intermediate and long types—these abnormalities will naturally go unnoticed at the time when further experiments might have served to elucidate the problems involved.

It may be mentioned that the arithmetic entailed by these reductions is somewhat long and tedious. Care has, however, been taken to avoid errors, and where unsatisfactory resolutions have been obtained the work has been re-done; nevertheless it is, of course, always possible that some slip in the arithmetic may have been overlooked.

Human Strain III.

The distribution of length-measurements of this strain was clearly different in type from that of the other human strains. Professor Pearson gives the constants of this distribution as—

$$\text{Mean} = 26{\cdot}172$$
$$\mu_2 = 23{\cdot}5226 \qquad \mu_3 = -37{\cdot}1323$$
$$\mu_4 = 1179{\cdot}3079 \qquad \mu_5 = -3248{\cdot}4381$$

Which led to two components, of which he writes:—"The graph while giving broadly some of the features of the case is by no means a satisfactory fit; for $n=21$ groups, $\chi^2=86$ and P is $<{\cdot}000,000,1$. The diagram suggests that we are probably dealing with a mixture of three components with means about 18·5, 25·5 and 31·0, but at present we have no satisfactory method of performing multiple resolutions of this character." This also appeared to me to be a probable solution for the distribution. The area left, after the first component of the solution by the nonic had been subtracted from the distribution, was submitted to the same method of analysis in order to see whether a satisfactory resolution could be obtained into two normal components. In order to give the tail of the distribution "higher contact" with the axis, which it clearly must have if it is to be represented by a normal curve, slight alterations were made in the proximate tail by subtracting a small area from the first component. The result was as follows:—

$$\text{Mean} = 28{\cdot}1370$$
$$\mu_2 = 11{\cdot}9493 \qquad \mu_3 = -2{\cdot}0722$$
$$\mu_4 = 341{\cdot}7133 \qquad \mu_5 = -60{\cdot}1526$$

Giving for the nonic:—

$$q^9 - 3{\cdot}0346q^7 + {\cdot}006441q^6 + 2{\cdot}9316q^5 - {\cdot}3169q^4 - {\cdot}8829q^3 - {\cdot}008{,}962q^2 + {\cdot}000{,}063{,}9q - {\cdot}000{,}000{,}08 = 0.$$

The root was $p_2 = 10q = -5{\cdot}9751$, leading to the components given in Table I. and shown in Chart IX. $P = {\cdot}28$.

A normal curve was then calculated for the remainder of the distribution. The three means are now at 18·7044, 24·5319 and 29·7893, giving for $n = 25$ groups, $P = {\cdot}08$. Thus, if this resolution is to be considered satisfactory, only once in about twelve trials should we expect as bad a fit as this. The observations are, however, rather irregular and it does not seem likely that any resolution could give a very satisfactory value to P. An inspection of the graph shows that the first component is a very bad fit; this area was, therefore, treated similarly to the other, with the following result:—

$\mu_2 = 1{\cdot}3892$ $\quad$ $\mu_3 = 0{\cdot}3080$
$\mu_4 = 5{\cdot}0917$ $\quad$ $\mu_5 = 2{\cdot}6955$

Reducing nonic:—

$$p_2^9 - 2{\cdot}4430p_2^7 + 0{\cdot}1424p_2^6 + 0{\cdot}3638p_2^5 - 3{\cdot}1059p_2^4 - 1{\cdot}8068p_2^3 + 0{\cdot}0173p_2^2 + 0{\cdot}0251p_2 - 0{\cdot}0009 = 0.$$

Which leads to $p_2 = -0{\cdot}6564$, and the components as given in Table I. and shown in Chart X. For the four components $P = {\cdot}25$. Thus the successive reductions have given increasing values for the probability of "goodness of fit" from $<{\cdot}000{,}000{,}1$ to ·08, and finally to ·25.

I do not think this can be taken as proof that the reduction is necessarily valid, but a study of the distribution seems to me to indicate that this is a very probable solution, and the conclusion that in this case we are dealing with a dual infection naturally suggests itself.

As in Strain II., this distribution was obtained from two rats, and the measurements are recorded separately. Here, however, the distributions from both sources are closely similar and nothing, therefore, is to be gained by treating them separately as was done with Strain II. Professor Pearson found for these two distributions— $\chi^2 = 14{\cdot}715$, giving $P = {\cdot}9038$. He writes:—"We should not hesitate to assert identity of strains and of treatment in the case of the trypanosomes from these two rats."

The fact that two rats gave closely similar results would tend to negative a suggestion that a mixed infection, due to an accidentally or naturally infected rat being used as intermediate host, might account for the hypothesized dual infection. In this case a dog was an intermediate host between man and the rats.

Human Strains IV. and V.

Professor Pearson had shown that these two strains could safely be considered together, as their distributions were closely similar;

he writes—"The correspondence is so close that we have combined Strains III. (*sic*, an obvious misprint for IV.) and V. for all other comparisons." These strains, therefore, were dealt with together, and give the following constants:—

Mean = 23·992
μ_2 = 8·1246 μ_3 = 5·0912
μ_4 = 166·4060 μ_5 = 231·7179

Reducing nonic:—

$$q^9 - 1{\cdot}10676q^7 + {\cdot}03888q^6 + {\cdot}08985q^5 - {\cdot}4130q^4 - {\cdot}1627q^3 + {\cdot}000{,}586{,}9q^2 + {\cdot}000{,}849{,}8q - {\cdot}000{,}017{,}42 = 0.$$

Which leads to $p_2 = 10q = -4{\cdot}3147$ and the components given in Table I. and shown in Chart XI. $P = {\cdot}85$.

Human Strain VI.

Mean = 21·70
μ_2 = 9·2667 μ_3 = 15·7620
μ_4 = 251·7159 μ_5 = 982·3631

Reducing nonic:—

$$q^9 - {\cdot}20641q^7 + {\cdot}37266q^6 - 2{\cdot}2484q^5 - 1{\cdot}9865q^4 + {\cdot}5404q^3 + {\cdot}5596q^2 + {\cdot}01456q - {\cdot}01533 = 0.$$

Leading to $p_2 = 10q = -5{\cdot}1390$ and the components given in Table I. and shown in Chart XII. $P = {\cdot}46$.

Human Strain VII.

Mean = 22·51
μ_2 = 13·6586 μ_3 = 28·6169
μ_4 = 436·4956 μ_5 = 2285·9717

Reducing nonic:—

$$q^9 - 4{\cdot}3111q^7 + 1{\cdot}2284q^6 - 8{\cdot}2416q^5 - 38{\cdot}4100q^4 - 19{\cdot}7937q^3 + 8{\cdot}1471q^2 + 3{\cdot}3042q - {\cdot}54921 = 0.$$

Here $p_2 = 10q = -8{\cdot}6657$, which leads to the components given in Table I. and shown in Chart XIII. $P = {\cdot}13$.

The somewhat unsatisfactory nature of this result is due largely to want of agreement in the one group at 22 microns.

Human Strain VIII.

Mean = 22·458
μ_2 = 7·4369 μ_3 = 19·0571
μ_4 = 223·6980 μ_5 = 1347·5668

Reducing nonic:—

$$q^9 + 2{\cdot}02214q^7 + {\cdot}54476q^6 + {\cdot}85331q^5 + 3{\cdot}8454q^4 + 2{\cdot}14503q^3 - {\cdot}17352q^2 - {\cdot}30481q - {\cdot}047901 = 0.$$

No negative root could be found for this equation, and unless there is some error in the arithmetic this distribution is not strictly capable of resolution into two normal components, nor can it be satisfactorily fitted by a single normal curve, as is shown in Chart XIV. It is probably multiple, consisting of more than two components.

Human Strain IX.

Mean = 21·198

$\mu_2 = 18{\cdot}5155$ $\quad$ $\mu_3 = 62{\cdot}6905$

$\mu_4 = 879{\cdot}5484$ $\quad$ $\mu_5 = 6306{\cdot}7863$

Reducing nonic:—

$$q^9 - 5{\cdot}2122q^7 + 5{\cdot}8952q^6 - 91{\cdot}3741q^5 - 319{\cdot}0017q^4 - 41{\cdot}0530q^3 + 368{\cdot}9150q^2 + 92{\cdot}0063q - 60{\cdot}7033 = 0.$$

Which leads to $p_2 = 10q = -13{\cdot}4000$ and thence to the components given in Table I. and shown in Chart XV. $P = {\cdot}36$.

Human Strain X.

Mean = 23·57

$\mu_2 = 8{\cdot}1298$ $\quad$ $\mu_3 = 5{\cdot}0964$

$\mu_4 = 174{\cdot}6051$ $\quad$ $\mu_5 = 335{\cdot}3301$

Reducing nonic:—

$$q^9 - {\cdot}8286q^7 + {\cdot}03896q^6 + {\cdot}08340q^5 - {\cdot}16056q^4 - {\cdot}04972q^3 - {\cdot}000{,}684{,}3q^2 + {\cdot}000{,}638{,}8q - {\cdot}000{,}017{,}5 = 0.$$

Here no real negative root of the equation appears to exist, and therefore no resolution into two suitable normal distributions results.

This distribution is not far from symmetrical, $\beta_1 = 0{\cdot}0483$ and $\beta_2 = 2{\cdot}6418$. A single normal curve does not give a bad general representation of this distribution. Chart XVI. $P = {\cdot}16$. The similarity between this distribution and those of Strains IV. and V. is shown by the value of the constants, and also by the value of χ^2 as obtained from the formula:

$$\chi^2 = S_1^s \left[\frac{N N_1 \left(\frac{f}{N} - \frac{f'}{N_1} \right)^2}{f + f'} \right]$$

Comparing strain X. with strains IV. and V., $\chi^2 = 18{\cdot}02$ and $P = {\cdot}26$. The satisfactory nature of the resolution in the case of strains IV. and V. contrasts curiously with the difficulty experienced in regard to strain X.

The evidence, therefore, for dimorphism, if judged by the analysis of this strain, is not very convincing and inspection of the

graph does not suggest a bi-modal distribution. Nevertheless, this was the strain chosen by the Commission with which to carry out some experiments on dimorphism.

Six rats, it is stated, were each inoculated with one trypanosome of this strain. Three successful results were obtained, but only two of them are recorded in the Report; these are referred to below as strain X, A and B. The Commissioners state (p 55)—"As will be seen from the following curves, from the single long and slender type, short and stumpy, intermediate and long and slender forms resulted." . . . "From these three experiments" (two only are given) "then, it may be concluded that in the dimorphic Trypanosome causing disease in man in Nyasaland, a single species is being dealt with." This conclusion does not seem to me to necessarily follow from the experiments recorded, without at least some further analysis. The fact that all forms are reproduced from one trypanosome does not surely prove the species dimorphic, if the standard deviation is not increased; the normal variability of a monomorphic type would lead to a similar phenomenon. The distribution of strain X.A is not, on inspection, obviously dimorphic, any more than strain X. itself was, whilst strain X.B is almost obviously so. On analysis, however, both are found to be bi-modal with similar positions for the modes; the relative areas of the two components are, however, dissimilar. Thus—

Human Strain X. A.

Mean = 22·242

$\mu_2 = 6{\cdot}2121$ $\quad\quad$ $\mu_3 = 5{\cdot}5693$

$\mu_4 = 106{\cdot}5572$ $\quad\quad$ $\mu_5 = 223{\cdot}2602$

Reducing nonic:—

$$q^9 - {\cdot}322473q^7 + {\cdot}04653q^6 - {\cdot}17320q^5 - {\cdot}16581q^4 - {\cdot}01767q^3 + {\cdot}005{,}668{,}4q^2 + {\cdot}000{,}354{,}6q - {\cdot}000{,}029{,}84 = 0.$$

And the root $p_2 = 10q = -3{\cdot}1014$, which leads to the components given in Table I. and shown in Chart XVII. $P = {\cdot}53$.

Human Strain X. B.

Mean = 23·760

$\mu_2 = 8{\cdot}3971$ $\quad\quad$ $\mu_3 = 1{\cdot}9125$

$\mu_4 = 161{\cdot}7553$ $\quad\quad$ $\mu_5 = 157{\cdot}5837$

Reducing nonic:—

$$q^9 - 1{\cdot}7422q^7 + {\cdot}005487q^6 + {\cdot}92742q^5 - {\cdot}03375q^4 - {\cdot}13988q^3 - {\cdot}00237q^2 + {\cdot}000{,}026{,}63q - {\cdot}000{,}000{,}049 = 0.$$

Whence the root $p_2 = 10q = -4{\cdot}3550$, which leads to the components given in Table I. and shown in Chart XVIII. $P = {\cdot}99$.

We may now consider what deductions can be drawn from the foregoing analyses of the human strains from Nyasaland. With the exception of strains II.B, III., VIII. and X. these strains are all capable of resolution into two normal components by mathematical analysis. The variation in position of the mean of the first component is from 18·3246 to 22·7695, and of the second component from 25·1799 to 27·7568 microns. Such variations are much too great to be accounted for by errors of sampling; to what, then, may they be attributed? Firstly, we may consider whether the variation is due to differences in technique in the preparation of the slides, and to personal error of the observers. This, I think, can be excluded as the cause, for the following reasons: if greater shrinkage took place on certain slides than on others it would not be likely to affect one strain more than another, because many slides, prepared on different days, were used in obtaining the distributions, and the values of the means of the two components are not correlated to any extent. If this were the explanation one would expect that the means of both components would be either in excess or defect of the average: it will be noted that a low value for one component is sometimes associated with a high value for the other. Secondly, the difference in the animals from which the strain was obtained for measurement cannot account for the difference, for only measurements obtained from rats have been dealt with.

It would seem possible, however, that the passage of strains through different mammalian hosts between man and the rat might influence the type of the distribution; a study of the components of the human strains must therefore be made in conjunction with the information in regard to previous passages of the trypanosome through animals, as recorded by the Commission on page 43 of the Report.

It will be noted that, in regard to the first component, strains II.B, IV. and V. show close similarity but differ considerably from the remaining human strains in regard to the position of the mean, the value being about 2 microns in excess of the mean of the remainder. This is also true both of the second component of these strains and of strain I. The first five of these human strains differ from the last five, and, if we exclude strain III. as being totally different in character, the variation in the two groups appears to consist in an increase of the modal length of the components. In the first group the dog was the intermediate host between man and the rat; in strains II., IV. and V. it was the only intermediate host; while in strain I. the measurements were obtained from several rats which had

been inoculated from—in one case a monkey, in another a dog, whilst in two others both a monkey and a guinea-pig were the intermediate hosts. This variation in previous treatment may account for the difference between strain I. and strains II.B, IV. and V.

In strains VI. and IX. the intermediate host was a monkey and both these strains are capable of resolution into two normal components.

In strains VII., VIII. and X. the blood from the human patient was inoculated directly into the rat from which the measurements were made. Strain VII. is capable of resolution into two components, but the fit of the resulting components to the original distribution is worse than that for any other bi-modal resolution in the table, and a study of the distribution appears to me to suggest that it is of a more complex character. The remaining strains, VIII. and X., have, so far as I can find, no suitable root to the nonics, and are not, therefore, strictly capable of resolution into two normal components. In the case of strain X. subsequent passage through rats from single trypanosomes resulted in distributions which resolved into closely similar components differing only in their relative areas.

It does not appear, therefore, that this investigation supports the contention of the Commission (p. 43) that "on comparing the curves of the ten strains, it cannot be said that the passage from dog to rat, or from monkey to rat, or direct from man to rat has had any marked influence on the character of the curve." It is no doubt true that simple inspection of the graphs does not reveal the differences, but it has already been pointed out that this method of examining the distributions is quite inadequate.

If the above conclusions are accepted it will be agreed that for measurement-distributions to be of value in guiding the observer to the differentiation of species of trypanosomes not only must a proper analysis of the distribution be made but the animal hosts through which the trypanosomes are passed must be the same. Further, there is a certain amount of evidence to suggest that passage through various animals may definitely modify the type and possibly eliminate a component of the distribution. The number of animal passages may also be a factor of importance.

The above enquiry, while providing a possible explanation of some of the characteristics of the components, as determined by analysis, has not thrown any light on two difficulties, namely, the difference between the strains II.A and II.B, and the peculiarities of strain III. It is possible that more detailed information as to intermediate hosts, etc., might have helped in elucidating the problems.

With respect to the results as they stand in Table I. the following points may be noted:—

(*a*) The mean of the first component of the Nyasaland human strains is rather larger than that of the other distributions given in the table, but this is due to the inclusion of strains II.B, IV. and V., which have means considerably in excess of the remaining strains in the group.

(*b*) The same remarks are true, but to a lesser extent, in regard to the position of the means of the second component.

(*c*) In both components the mean of the coefficients of variability is smaller for the Nyasaland human strains than for the remainder of the strains. The Nyasaland game strains (Wild Game strain, Mzimba strain and Liwonde strain) have, however, coefficients of variability for their first component more closely comparable with the Nyasaland human strains than with the other members of the group, but the dispersion of the second component in the first two examples referred to is considerably greater than that of the Nyasaland human strains.

(*d*) It is curious to note that there is a closer agreement between the components of *T. rhodesiense* and *T. gambiense* (excluding the abnormal *gambiense* strain discussed later) than between *T. rhodesiense* and the Nyasaland human strains. This may be an artificial effect due to intermediate hosts, treatment of slides, etc., but sufficient details are not available to enable a valid opinion to be formed as to the cause.

(*e*) The two examples of *T. rhodesiense* are from the same strain. In reply to an enquiry on this point Professor J. W. W. Stephens kindly writes—"there is *only one* strain of *T. rhodesiense* in question, which was originally obtained from man; the counts were made from subinoculation in rats."

(*f*) It seems possible, in the light of some experiments carried out by the Commission in regard to the "Naturally-infected Dog Strain," that the percentage of trypanosomes in each group may be a function of the age of the strain in the rat, but whether this difference in ratio of number in each group, even if confirmed, be correlated with a shifting in the position of the mean is a further point for investigation. The Commission writes (p. 95)—"The short and stumpy and the intermediate forms have almost disappeared, and only the long and slender survive." . . . "The percentage of the long and slender gradually increases until, at the end of 17 passages" (through rats) "it reaches 100%." The estimations are based simply on inspection of the distributions and no graph showing a unimodal distribution is given in the report. The following extract (p. 82) does

not, however, support this view:—"It has usually been thought that a trypanosome kept under laboratory conditions, and without the opportunity of passage through its invertebrate host, the tsetse fly, would tend to change in morphology. These curves, on the other hand, show that a trypanosome, after passage through horses and cattle for some years—exact time unknown—is unchanged by three passages through its invertebrate host, *G. morsitans*." No attempt is made to reconcile these divergent statements. In regard to the latter the experiments were carried out with *T. brucei* and the evidence in support of the opinion expressed does not seem to me to warrant the conclusion drawn, but the point will be referred to again when strains of *T. brucei* are considered.

(*g*) An inspection of the histograms of these strains will show how greatly the general appearance may vary. In some cases there is very little evidence, on inspection, of bi-modality and the distribution may be fairly well represented by a single normal curve, while in others the compound nature of the distribution is evident at a glance.

In view of the difficulty of resolving the Nyasaland human strain III., and the result obtained, the Tanganyika strain of *T. gambiense* II., the histogram of which had a somewhat similar general appearance, was analysed.

T. gambiense, Tanganyika Strain II. (rat).

Mean = 25·85

$\mu_2 = 24{\cdot}8322$ $\mu_3 = 45{\cdot}6347$

$\mu_4 = 1195{\cdot}4210$ $\mu_5 = 4017{\cdot}1344$

Reducing nonic:—

$$q^9 - 22{\cdot}9071q^7 + 3{\cdot}12378q^6 + 60{\cdot}4893q^5 - 653{\cdot}4701q^4 - 1246{\cdot}5155q^3 - 25{\cdot}6129q^2 + 113{\cdot}5379q - 9{\cdot}0317 = 0.$$

Whence $p_2 = 10q = -19{\cdot}4366$, and the components are as given in Table I. and shown by the dotted lines in Chart XIX. Here $P = {\cdot}01$, which is not a satisfactory value. It is clear, however, that the first component does not even approximately represent the observations. The area of the original distribution not represented by the second component (with slight alteration at the tail), was therefore analysed

The reducing nonic is:—

$$q^9 - 1{\cdot}0293q^7 + {\cdot}12252q^6 - {\cdot}48418q^5 - 1{\cdot}11130q^4 - {\cdot}30521q^3 + {\cdot}04750q^2 + {\cdot}00785 - {\cdot}00054 = 0.$$

Whence $p_2 = 10q = -4{\cdot}8305$, leading to components as given in Table I. and shown in Chart XIX.

The value of P for goodness of fit of these components with the second component of the first reduction is ·82. Although this result is satisfactory the distribution represented by the second component of the original reduction does not appear to be properly represented by a single normal curve; this area was therefore treated as the former.

The reducing nonic is:—

$$q^9 - \cdot 79520q^7 + \cdot 00440q^6 + 1\cdot 8540q^5 - \cdot 14225q^4 - \cdot 15875q^3 - \cdot 00373q^2 + \cdot 000{,}078q - \cdot 000{,}000{,}025 = 0.$$

Whence $p_2 = 10q = -2\cdot 4753$ and the components are as given in Table I. and shown in the chart. For the four components $P = \cdot 99$. Thus the final resolution arrived at resembles that for Nyasaland human strain III., and a similar explanation may account for the result. Unfortunately, however, sufficient details as to the history and treatment of the strains is not given in the report to enable one to form much of an opinion as to the soundness or otherwise of the hypothesis suggested. These reductions are, however, further evidence in regard to the uselessness of mere inspection of graphs of such distributions for purposes of classification.

An example of the wild game strain and the Mzimba strain has been analysed by Professor Pearson and the results are quoted in Table I. An additional analysis of such a strain is here given; the strain was chosen because it did not exhibit any feature of bi-modality on inspection.

Liwonde Strain XXII.

Mean = 22·986

$\mu_2 =$ 5·0345	$\mu_3 =$ 1·2030
$\mu_4 =$ 69·3936	$\mu_5 =$ 19·2695
$\beta_1 =$ 0·0113	$\beta_2 =$ 2·7379

These constants show that the distribution is nearly symmetrical. On inspection of the histogram there is no evidence of bi-modality, and it is well fitted by a single normal curve, as will be seen on reference to Chart XX. $P = \cdot 62$.

The reducing nonic, however, is:—

$$q^9 - \cdot 23254q^7 + \cdot 002171q^6 + \cdot 000987q^5 - \cdot 01520q^4 - \cdot 001826q^3 + \cdot 000{,}005{,}32q^2 + 000{,}000{,}557q - 000{,}000{,}003 = 0.$$

Which gives the root $p_2 = 10q = -1\cdot 2554$ and the components shown in Chart XX. $P = \cdot 95$. So that, although one was almost justified in regarding this example as unimodal, analysis has, however, demonstrated the compound nature of the distribution.

T. brucci Strains.

The resolution of strains of *T. brucei*, for some reason or other, appears to give rise to peculiar difficulty. Professor Pearson in discussing a strain of this trypanosome remarks:—"But the evidence for dimorphism has largely disappeared." No suitable root for the reducing nonic could be found. In two of the five distributions of *T. brucei* dealt with below the reducing nonic had apparently no suitable root, and the solution obtained in a third cannot be considered as satisfactory. There are difficulties, moreover, in regard to the interpretation of the solution in another example. These four examples are all from strain No. XXV. and the only satisfactory resolution is for Zululand strain 1896. This latter, however, gives components with means markedly different from any which could possibly be obtained as a solution to the distributions of strain XXV.

T. brucei XXV.—The original strain before passage through *G. morsitans.*

Mean = 20·832

$\mu_2 =$ 3·7404 $\quad \mu_3 =$ 4·7279

$\mu_4 =$ 55·0186 $\quad \mu_5 =$ 209·3757

$\beta_1 =$ 0·4271 $\quad \beta_2 =$ 3·9325

Reducing nonic:—

$$q^9 + \cdot45660q^7 + \cdot03353q^6 + \cdot10996q^5 + \cdot04601q^4 - \cdot000535q^3 - \cdot00203q^2 - \cdot000261q - \cdot000,011,17 = 0.$$

No suitable root could be found, nor was a possible solution obtained by putting trial values for p_2 in the equation for p_3. The distribution, although it does not show any marked feature of bi-modality, is not, as can be seen from the values for β_1 and β_2, satisfactorily represented by a single normal curve. $P = \cdot002$, Chart XXI.

This distribution is similar to those stated to be obtained from the naturally-infected dog strain, after many passages through rats, in that it appears on inspection to have lost its dimorphic character. In this case, however, it is the smaller component, the short and stumpy type, which has persisted instead of the long and slender type as in the dog strain. This apparent disappearance of the dimorphic character of the trypanosome with age and frequent animal passage does not seem to be a constant phenomenon.

The next example is of a strain of *T. brucei* very much older than that just discussed; it is nevertheless markedly dimorphic in character.

T. brucei, Zululand Strain 1896.

Mean = 22·515

μ_2 = 36·2464 μ_3 = 69·9189

μ_4 = 2213·8431 μ_5 = 9786·8190

Reducing nonic:—

$$q^9 - 60{\cdot}465q^7 + 7{\cdot}333q^6 + 792{,}89q^5 - 3377{\cdot}43q^4 - 13969{\cdot}44q^3 - 2234{\cdot}76q^2 + 1651{\cdot}48q - 116{\cdot}83 = 0.$$

The root $p_2 = 10q = -30{\cdot}5770$, which leads to the components as given in Table I. and shown in Chart XXII. $P = {\cdot}44$.

To return to a consideration of strain XXV.—This strain was passed through *G. morsitans* three times.

T. brucei XXV.—After first passage through *G. morsitans.*

Mean = 22·366

μ_2 = 10·5887 μ_3 = 22·3631

μ_4 = 304·0286 μ_5 = 1478·8279

Reducing nonic:—

$$q^9 - 1{\cdot}1317q^7 + {\cdot}75016q^6 - 5{\cdot}5731q^5 - 8{\cdot}9208q^4 + {\cdot}07013q^3 + 2{\cdot}8460q^2 + {\cdot}32348q - {\cdot}12508 = 0.$$

Which gives a root $p_2 = 10q = -6{\cdot}4042$, which leads to the components given in Table I. and shown in Chart XXIII. $P = {\cdot}015$. This resolution, therefore, cannot be considered satisfactory, and an inspection of the graph will show that no two normal components could be expected to properly represent the distribution. The passage of this trypanosome through the tsetse fly has apparently profoundly modified the type, the distribution being now clearly multimodal. The explanation of this change, however, is not evident, as further passages through the fly result in an apparent loss of this character.

T. brucei XXV.—After second passage through *G. morsitans.*

Mean = 19·73

μ_2 = 5·1578 μ_3 = 3·4670

μ_4 = 83·9936 μ_5 = 140·8048

β_1 = 0·0851 β_2 = 3·1710

In this example, as the values of the β's show, a single normal curve will give a fair representation of the distribution. Fitted with a normal curve as shown in Chart XXIV. $P = {\cdot}19$.

The reducing nonic is:—

$$q^9 + {\cdot}14651q^7 + {\cdot}01793q^6 - {\cdot}03297q^5 - {\cdot}001583q^4 + {\cdot}004686q^3 + {\cdot}0004148q^2 - {\cdot}00002377q - {\cdot}000001697 = 0.$$

In this case there were two possible roots, $p_2 = 10q = -1{\cdot}1020$ and $p_2 = 10q = -0{\cdot}5019$. In the former case $P = {\cdot}75$ and in the latter $P = {\cdot}88$. Excepting that the means of the components give such unusual values it would seem that the latter should be accepted as the correct analysis. It must be admitted, therefore, that there is some difficulty in interpreting the analysis of this distribution; it appears to be bi-modal but is obviously different in type from that obtained from the first passage through *G. morsitans*. The two sets of components are given in Table I. and shown in Charts XXV. and XXIV.

T. brucei XXV.—After third passage through *G. morsitans*.

$$\text{Mean} = 21{\cdot}194$$

$$\mu_2 = 5{\cdot}0290 \qquad \mu_3 = 5{\cdot}3953$$

$$\mu_4 = 78{\cdot}3542 \qquad \mu_5 = 253{\cdot}0555$$

$$\beta_1 = 0{\cdot}2289 \qquad \beta_2 = 3{\cdot}0981$$

The reducing nonic is:—

$$q^9 + {\cdot}08683q^7 + {\cdot}04366q^6 - {\cdot}02727q^5 + {\cdot}01085q^4 + {\cdot}011286q^3 + {\cdot}000{,}814q^2 - {\cdot}000{,}084{,}08q - {\cdot}000{,}024{,}67 = 0.$$

No suitable root to this nonic could be found, nor was any solution obtained by putting trial values for p_2 in the equation for p_3. The general character of the distribution is moderately well represented by a single normal curve, although the fit is not good. $P = {\cdot}06$. Chart XXVI.

The Commission sums up the results of these experiments as follows (p. 82)—"From this curve" (*i.e.*, after third passage) "it will be seen that three passages through *G. morsitans* has had little or no effect in changing the character of this trypanosome as regards distribution of length."

It seems to me that, even if we ignore the distributions after first and second passages, this conclusion is not justified on the evidence submitted. It is true that the distribution after third passage has some general resemblance to that obtained before passage through the tsetse, but even if judged by inspection—regarding the population as homogeneous—it is 140 to 1 against the distributions being derived from the same population. If, on the other hand, we take into account the evidence of the effect of the previous passages through the fly it seems clear that passage through the tsetse very profoundly modifies the type. The apparent similarity may, therefore, be simply a coincidence and even mask a fundamental difference in type. The statistical resolutions are unfortunately inconclusive, and

it does not seem to me wise with the limited data at present available to draw any conclusions as to the effect of passage through the tsetse fly. The fundamental type of the distribution, by which expression I refer to the position and character of the components and not to their relative areas, may or may not be altered, but until this strain is shown to be truly mono-, di- or multi-morphic no satisfactory answer will, I believe, be obtainable. At present, unfortunately, there are mathematical difficulties to be overcome before a definite opinion on this point can be given.

Professor Pearson in summarising the results of his investigation into the statistical analysis of dimorphic trypanosome distributions writes—" I think we may conclude that, allowing for the errors of random sampling and the errors arising from the resolving process, the deviations observed in the variability of our two components do not invalidate the hypothesis—

(1) That the widely divergent results obtained from different strains are due to the existence in the same individual of two types of trypanosome with very varying percentages from individual to individual.

(2) That one of these types has a mean length of about 19·8 microns and a variability of about 1·8 microns, the other a mean of about 26·0 microns and a variability of about 3·0 microns. The means may vary one or two microns with the nature of the host and the variability 0·5 to 1 micron."

We may ask, how far does the present enquiry bear out these conclusions? In regard to the first point there seems no doubt that the divergent results, not only in regard to different strains but in different samples of the same strain, are largely due to this cause. The position of the means of the two components, as given above, is confirmed by these further analyses; but the variability both of the means and standard deviations is rather larger than that suggested. There is also some reason for thinking that not only the animal from which the count is made, but the previous mammalian hosts may affect these measurements.

At present these analyses are too few in number, and the treatment of the trypanosomes, by animal passages and so forth, has not been sufficiently uniform to enable any definite conclusions to be drawn in regard to classification by this morphological feature. There are, however, certain indications which suggest that there are morphological differences between the Nyasaland human strains, the Nyasaland game strains, and the Zululand *T. brucei* strains.

The coefficient of variability in the human strains does not differ much in the two components; either may be the larger, although on the average that with the greatest mean has a slightly greater variability. In the Nyasaland game strains the second component has a very considerably greater variability than the first, while apparently this feature tends to be reversed in the *brucei* strains. The means of the components of the human strains tend to be a little greater than those of the game strains; this is most marked in respect to the second component of the Nyasaland game strains. The want of correspondence between the variability of the Nyasaland human strains and that of the two samples of the human strain, *T. rhodesiense*, is striking. This may be accounted for either by different treatment or age; if the latter be the explanation, in whole or in part, it is another example of the importance of uniformity of treatment if measurements are to be of value for classification.

It is, I think, impossible with our present knowledge to form any sound judgment as to whether these differences are merely accidental, or whether their existence would be confirmed when further examples had been analysed; and if confirmed whether such differences are sufficient to warrant the groups being considered as distinct species.

It seems probable, however, that length-measurements, if submitted to proper analysis, *may be* of great value in the study of trypanosome strains. For the present it is possible that their chief use would be to indicate difficulties and give hints as to the lines of investigation necessary for their solution. When more information has been accumulated the measurements themselves may become of intrinsic value for purposes of classification. On the other hand, if these measurements are used without suitable analysis they are without any scientific value, and we can endorse Yorke and Blacklock's conclusion, previously quoted, that "the character of the biometric curves affords no assistance in diagnosis." It would be no more just, however, to abandon this method of research as useless because a technical analysis is required for its interpretation than it would be for the public health official to give up the collection of samples of drinking water because inspection of the water gives no safe guide to its chemical and bacterial qualities.

There are undoubted difficulties in the interpretation of some of these analyses, but it is possible that some or even many of the points at present obscure might be elucidated if all the circumstances were known. Neither the difficultes nor the laborious character of the

arithmetical work justify our abandoning any line of investigation which may be of assistance in solving the important problems of trypanosome infections.

This incomplete investigation has, however, demonstrated that there is no scientific justification for classifying as identical, from length-measurements, the *T. brucei*, the *T. rhodesiense*, the human Nyasaland strains, and the Nyasaland wild game strains, unless indeed all the dimorphic strains, including *T. gambiense*, are to be considered merely as variants of one species of trypanosome. As above stated, there are some reasons for regarding them as probably different, but until uniformity of treatment is adhered to it seems unlikely that much real information will be obtained from this method of investigation.

2. "Have they the same habits and actions when alive?"

This is a question with which the Commission deals very briefly; it states—"They" (*T. brucei* and *T. rhodesiense*) "are also alike in habits and actions when alive." Unless, however, other dimorphic trypanosomes differ from these species in habits and actions their similarity in this respect can be of little value for purposes of classification.

3. "Do the trypanosomes infect the same animals and kill them in the same time?"

In regard to this method of differentiating the species of trypanosomes the Commission has carried out a considerable amount of work, the chief results of which may be briefly summarized in the following tables:—

TABLE II.

Average number of days' illness before death.

Strain.	Goat.	Monkey.	Dog.	Rat.	Rabbit.	Guinea-pig.
Human	42	26	24	30	28	67
Wild Game	46	38	41	32	—	—
G. morsitans... ...	54	38	29	26	47	81
T. brucei...	77	29	18	27	33	44
Liwonde	33	41	28	26	—	84
Nat. Inf. Dog	R	R	37	48	182	R
T. gambiense ...	R	159	96	137	—	264

TABLE III.

Percentage of recoveries in infected animals.

Strain.	Goat.	Monkey.	Dog.	Rat.	Rabbit.	Guinea-pig.
Human	0	0	0	0	0	0
Wild Game	0	0	0	0	—	—
G. morsitans ...	6	7	4	0	0	0
T. brucei	0	0	0	0	0	0
Liwonde	0	0	0	0	0	0
Nat. Inf. Dog ...	100	100	21	0	0	100
T. gambiense ...	100	64	0*	23	—	0*

*A number of experiments negative—*i.e.*, inoculated animals did not develop trypanosomes. The four dogs which showed trypanosomes all died; out of 14 guinea-pigs inoculated only one developed trypanosomes, and it died.

Among the conclusions drawn from these results by the Commission are the following:—"The pathogenic action on various animals of the Human Strain, the Wild Game Strain, and the Wild *G. morsitans* Strain is so much alike, that it may be concluded that they all three belong to the same species of trypanosome." (p. 115.)

"The pathological action on various animals of the Liwonde Strain is so much like that of the Human Strain that it may be concluded that these two belong to the same species of trypanosome." (p. 119.)

"The pathogenic action of *T. brucei*, Zululand 1913 Strain XXV., on various animals is so similar, not only in regard to the symptoms during life but also in the *post-mortem* appearances and rate of mortality, to that of the trypanosome causing disease in man in Nyasaland, that it affords another *proof*" (the italics are not in the original) "that these two trypanosomes are identical." (p. 125.)

"The Commission is of opinion that this" (the Naturally-infected Dog Strain) "is an aberrant or exceptional variety of strain of the Trypanosome causing disease in Man in Nyasaland—*Trypanosoma brucei* vel *rhodesiense*." (p. 134.)

All the strains, therefore, quoted in Table II., with the exception of *T. gambiense*, belong, in the opinion of the Commission, to the same species, which it terms *T. brucei* vel *rhodesiense*. If different strains of the same species can show such differences as are exhibited by the naturally-infected dog strain and, say, the *T. brucei*, clearly great caution must be exercised before it is safe to draw unsupported conclusions from this method of differentiating species. In regard to

goats, monkeys, and guinea-pigs there is a greater difference between the dog strain and the remaining Nyasaland trypanosomes than between the *T. gambiense* and this strain. It is somewhat curious, if this naturally-infected dog strain is really only an aberrant strain of *T. brucei*, that it should show so little difference in regard to its virulence, as judged by days-of-illness before death, in the dog and to a lesser extent in the rat, and yet so marked a difference with regard to the other animals against which it was tested.

If, however, smaller differences in virulence are to be considered of importance, then it seems doubtful whether we ought to consider identity established when we find it stated that *T. brucei* kills goats on the average in 77 days, the human strain in 42 days, and the wild game strain in 46 days. Although many groups in these tables are small, and therefore subject to errors of sampling to a marked degree, these particular differences are not due to errors of small samples. The mean values are significantly different: for example, the mean for wild game strain is 46 ± 5, and for *T. brucei* 75 ± 11. (The 77 given in the table by the Commission may be a misprint for 75, or a misprint may have occurred in the table of individual cases; the mean of the individuals in the group as recorded is 75.) Further, for the dog as host *T. brucei* gives a mean of 19 ± 0·6, and wild game strain 41 ± 2·7. Differences so great as this would not presumably occur owing to errors of sampling.

It appears to me, therefore, that this method of classifying species must be used with caution and that the results in the present state of our knowledge can only be interpreted in conjunction with other methods of investigation.

From the data given by the Commission and (excluding *T. gambiense*) reproduced in Tables II. and III., the reply to the question heading this section may, I think, be given thus:—These trypanosomes do not always infect the same animals, nor kill them in the same time. Excluding the naturally-infected dog strain, the reply to the latter clause of the question is also still in the negative. There are statistically significant differences in the time which these trypanosomes take to kill animals, but whether these differences are sufficient to establish difference of species is a further question, and the other methods of investigating the problem must be examined before any definite conclusions can be drawn. Nor does it seem to me that even if entire similarity in this respect were established should we be justified in claiming "absolute identity" for the strains if they did not show agreement in other characters. Yorke and Blacklock, in their paper already referred to, write in this connection—"It is obvious, therefore, that great caution must be exercised in expressing

an opinion as to what is the species of a given trypanosome from the length of time which it takes to kill laboratory animals. This is so at least in the case of trypanosomes maintained by artificial passage through experimental animals." It would seem, therefore, that in regard to this method of investigation, as in that dealing with morphological characters, uniformity of previous treatment of the strains is an essential factor if the results are to be capable of useful interpretation.

4. "Have they the same carrier? and does their growth or development in the carrier follow the same course?"

This is the fourth method by which it is suggested similarity of species may be tested. In regard to the first question, it has been shown that *T. rhodesiense* may be carried by *G. morsitans* and the Commission hold also by *G. brevipalpis*. *T. brucei* is apparently carried by *G. morsitans*, although in the original work of Sir D. Bruce in Zululand an error apparently occurred in the classification of the tsetse studied by him. Austin (Handbook of the Tsetse Fly—page 56) writes—"The species of Tsetse used by Sir David Bruce in Zululand, in 1895-96, in the course of his epoch-making researches into the etiology of Nagana now proves to have been *Glossina pallidipes*." Yorke and Blacklock write—"It was originally thought that a given trypanosome could be transmitted by one particular tsetse only. This is now known not to be so, *T. gambiense*, for example, can complete its development in both *G. palpalis* and *G. morsitans*." The Commission also showed that *T. gambiense* can complete its development in the *G. morsitans*. Further, *G. morsitans* is known to be the carrier of other varieties of trypanosomes. The fact, therefore, that two strains are carried by the same tsetse fly can be of little value in assisting classification.

In regard to the second part of the question, the Commission has shown that the cycle of development in the *G. morsitans* is similar for *T. brucei* and *T. rhodesiense*, in that the early stage takes place in the gut, and the later stage in the salivary glands of the fly. But this is also true of the *T. gambiense*; so that unless all three species of trypanosomes are to be assumed identical little weight can be placed on this similarity between *T. brucei* and *T. rhodesiense* in furtherance of their assumed identity.

5. "Cross-inoculation experiments. If an animal has recovered from an attack of disease set up by one of the two trypanosomes, is it immune or non-immune to infection by the other?" At first sight one might expect that this method should yield most valuable results in assisting to classify the various strains or species of trypanosomes;

there are, however, considerable practical difficulties to be overcome. The Commission has carried out some experiments in this connection with the Naturally-infected Dog strain and the Wild *G. morsitans* strain in relation to the Human Nyasaland strain and *T. brucei*. Their conclusions (p. 174) are as follows—

"The Naturally-infected Dog strain does not protect animals from the Human, Wild *Glossina morsitans*, and Zululand, 1913, Strains."

"The Wild *Glossina morsitans* Strain does not protect against the Human Strain."

Further, neither the wild dog strain nor wild *G. morsitans* strain protected animals against *T. brucei*.

The naturally-infected dog strain was shown to be unlike any of the strains previously dealt with by the Commission, so that although it is classified by them as *T. brucei* vel *rhodesiense* this appears to be against the weight of the evidence. So far, therefore, as these experiments go they appear to indicate that the naturally-infected dog strain and the wild *G. morsitans* strain are distinct from human strains IV. and V. and also from *T. brucei* in their immunilogical properties. In the absence, therefore, of definite proof that these strains are members of the same species this evidence gives rise to serious doubt as to whether they are even closely allied species. If they should prove to be but different strains of the same species some explanation of their difference in this respect will be required.

Much work on this aspect of the problem has been carried out by Laveran, and he considers that he has established the fact that the *T. rhodesiense* cannot be identified with *T. brucei*. Laveran et Nattan-Larrier (Abstract—Sleeping Sickness Bureau, Vol. IV., page 54)—"an experiment is cited in which a ram with immunity for Nagana showed infection after inoculation with *T. rhodesiense*. It is concluded that *T. rhodesiense* cannot be regarded as identical with either *T. gambiense* or *T. brucei*." In a later paper Laveran describes other experiments (Abstract—Sleeping Sickness Bureau, Vol. IV., page 135)—"The ram and sheep which had acquired immunity for *T. brucei* were inoculated with *T. rhodesiense* . . . . both animals had an acute infection. The conclusion is that *T. rhodesiense* is not the same as *T. brucei*."

Mesnil and Leger (Abstract—Sleeping Sickness Bureau, Vol. IV., page 211) have carried out active immunity experiments in regard to *T. gambiense* and *T. rhodesiense*. Their results indicated that a closer relationship exists between *T. gambiense* and *T. rhodesiense* than between *T. brucei* and either of the forementioned.

These immunity experiments therefore fail to support the contention that *T. brucei* is closely related to *T. rhodesiense*, much less that they are identical.

6. "Experiments on the effect of various sera."

A few experiments on the effect of sera on the various strains of trypanosomes were carried out by the Commission, the results of which are summed up as follows (p. 165)—"It must be confessed that very little can be concluded from these serum experiments. They are too few in number, and are only given here to act as a slight basis for future work. The evidence supplied by these few experiments is in favour of *T. brucei*, Zululand Strain, and the Wild *G. morsitans* Strain, Nyasaland, belonging to the same species, and *T. brucei* vel *rhodesiense*, Human Strain, Nyasaland, belonging to a different species."

The trypanolytic action of sera has been studied by Leger and Ringenbach (Abstract—Sleeping Sickness Bureau, Vol. IV., page 125). They found that serum from an animal infected with *T. brucei*, Zululand Nagana, had no action on *T. rhodesiense*, but that a slight effect was observed with sera from animals infected with *T. gambiense*, indicating a closer relationship of *T. rhodesiense* to *T. gambiense* than to *T. brucei*—thus bearing out Mesnil and Leger's results from experiments in acquired immunity.

7. "Geographical Distribution." In this connection the authors of the report write (p. 37)—"The Commission thinks that, in addition to the morphology and action on animals, the geographical distribution of 'Nyasaland sleeping sickness,' or '*Trypanosoma brucei* disease,' is the only way—experiment on man being impracticable—of showing that *Trypanosoma brucei* and *Trypanosoma rhodesiense* are one and the same." Up to this stage of the enquiry, in spite of the definition of *T. brucei* already referred to, the report leads one to assume that the trypanosome of the Nyasaland Sleeping Sickness was regarded as *T. rhodesiense*, and that the problem to be solved was whether this trypanosome is identical with *T. brucei*—that is the trypanosome of Nagana in Zululand. Here, however, Nyasaland Sleeping Sickness is apparently defined as "*T. brucei* disease," and it now appears that we are asked to compare the organism of Nyasaland Sleeping Sickness with *T. rhodesiense*. It is not, therefore, quite clear what the Commission really means by this sentence, but I am inclined to think the expression "*T. brucei* disease" should here be disregarded.

If, then, we may read the above quotation as meaning that the Commission considers that the geographical distributions of *T. brucei*

and of the trypanosome causing Sleeping Sickness in Nyasaland may throw some light on the question of their identity or otherwise, surely only the species definitely known to be *T. brucei* must be included by this term. It may be possible, or even probable, that the wild game strain of Nyasaland is identical with *T. brucei*, but there is no proof at present that it is so. Moreover, immunilogical experiments appear to negative such a proposition, even if the animal inoculation experiments can be accepted in its support. And further, we have, at present, no proof that the Nyasaland wild game strain is identical with the human strain.

Viewing this question with no presuppositions as to the identity of these strains the geographical distribution lends no argument in its support. *T. brucei* is very common in the southern East Coast districts of Africa, and yet a human infection is unknown here. Although carried out by nature, this is surely a very extensive "experiment on man," and one which cannot be fairly dismissed, as is done by the Commission, by assuming that cases do occur but are diagnosed as "Malaria."

Even if an occasional case of trypanosomiasis does develop in Zululand, man must evidently be very resistant to *T. brucei*, and one might expect therefore that such cases would probably be of a chronic type. If the organism of Nyasaland Sleeping Sickness is *T. brucei* it is somewhat surprising that in many geographical districts in which it abounds this trypanosome so rarely, if ever, affects man; and that where it does so, the incubation period is short and the progress of the disease exceptionally rapid and, so far, invariably fatal. There might, of course, be some explanation of such a phenomenon, but it is one of the facts which must be put in the scale when weighing the evidence for and against this hypothesis.

The history of the appearance and spread of the Nyasaland Sleeping Sickness suggests much more strongly that the disease has extended from the districts infected with *T. gambiense* than that it came from the South. This argument will have little weight with those who believe that Sleeping Sickness is an old-established disease in Rhodesia and Nyasaland.

It does not seem to me, therefore, that without making assumptions which are unjustifiable—in that they beg the question in dispute—any valid argument in favour of the identity of these two strains from the geographical distribution of their trypanosomes has been advanced by the Commission. Unless it is shown that human trypanosomiasis is contracted in Zululand, and is due to the *T. brucei*, a strong presumptive argument remains against the hypothesis of their identity.

Conclusions.

1. That the analysis of length distributions shows that the dimorphic trypanosomes dealt with, namely, *T. rhodesiense*, *T. brucei*, *T. gambiense*, Trypanosomes of Nyasaland Sleeping Sickness, and Nyasaland wild game strain, have certain features in common. With some exceptions, which are not confined to any particular strain, the general character of resolution is similar.

2. That there is some evidence to suggest that there is less similarity between the Nyasaland human strain and *T. brucei* than between *T. rhodesiense* of Stephens and Fantham and *T. gambiense* as recorded by these authors.

3. That the analysis suggests that uniformity in treatment of strains, prior to the inoculation of rats, from which measurements are made, may be an important factor in determining the mean size and the variability of the components of the distributions.

4. That at present no valid argument in regard to the identity or otherwise of *T. brucei* and the trypanosome causing disease in man in Nyasaland can be drawn from length-measurement-distributions.

5. That this method of investigation may probably prove of service in the study of trypanosome species when proper analytical methods are employed at the time the investigations are being undertaken, but that nothing of value can be expected from an uncritical examination, or mere inspection, of the crude distributions.

6. That at present the only valid argument in favour of the identity of the trypanosome causing disease in man in Nyasaland with *T. brucei* is to be drawn from experiments in regard to their pathogenicity for animals. There is some doubt, however, as to what weight should be attached to differences in period of illness before death, and, moreover, whether any stress should be laid on similarity in this respect in the absence of confirmation by other methods of investigation.

7. That the immunilogical experiments carried out by the Commission and others negative the suggestion of identity of *T. brucei*, Nyasaland human strain, Nyasaland wild *G. morsitans* strain, and *T. rhodesiense*.

8. That such serological experiments as have been undertaken negative the supposition that the *T. brucei* is identical, or even closely allied, to the trypanosome causing Sleeping Sickness in man.

If we may judge by the analogy of recent bacteriological investigations, it is probably to immunilogical and serological experiments that final appeal must be made to definitely establish the identity or otherwise of trypanosome strains. These negative experiments are, therefore, of great importance, and cannot be dismissed lightly in favour of a doubtful similarity of length-measurement-distributions, more especially when the assumed similarity has been obtained by unreliable methods.

9. That there is no evidence at present from the known geographical distribution of *T. brucei* to suggest that it is a cause of Sleeping Sickness in man; and that even if it were found to produce disease in man this would of itself constitute no greater argument in favour of its identity with the Nyasaland human trypanosome than with *T. gambiense*.

10. From the foregoing it would appear, therefore, that there is very little scientific evidence to support the contention that *T. brucei* and the trypanosome causing Sleeping Sickness in Nyasaland are the same species, while there are some very valid reasons for regarding them as distinct. Although the question may still be considered *sub judice* there does not seem to be any justification for entertaining a bias in favour of their identity. If this be admitted it follows that the recommendations of the Commission, which are based on their assumed identity, do not rest on a sure foundation of fact.

It is no doubt true that any nomenclature which helps to simplify our existing terminology is an undoubted gain. Incorrectly to multiply the number of strains of trypanosomes is to create confusion, but wrongly to classify distinct strains as identical is, it seems to me, a much more serious error; and to call them by the same name whilst there is any doubt as to their identity is bound to produce confusion and retard the necessary investigations.

In the course of this study several hypotheses have suggested themselves as possible explanations of some of the facts; it may perhaps be permissible to refer to one or two here.

If the Nyasaland human trypanosome is a new-comer into this country how are we to account for its presence? It should be borne in mind that Nyasaland Sleeping Sickness is a much more virulent or active disease than that of the more Northern territories associated with *T. gambiense*. This seems to be an argument against the disease being caused by an organism to which man is usually very resistant, such as *T. brucei* and probably also the wild game strains of Central

Africa. If then for the present we abandon this suggestion, not as being impossible but as *a priori* unlikely, the following are some of the alternatives:—

1. That the Nyasaland disease is caused by the *T. gambiense* and that the increased virulence is due to the change of the invertebrate host. As the *G. morsitans* is not the accustomed carrier for this trypanosome, possibly only very vigorous strains can complete their development in this host, and undergo some modification in consequence of the novel environment. In favour of such a suggestion the serological affinity shown, by Leger and Ringenbach, to exist between *T. rhodesiense* and *T. gambiense* can be adduced. I am not aware, however, that animal experiments bear out the suggestion that strains of *T. gambiense* which have been passed through *G. morsitans* are selected in respect to virulence. Against the possibility of the Nyasaland human trypanosome being a derivative of *T. gambiense* is the difference in its pathogenicity to animals.

2. A second alternative is that the human strain is derived from the wild game strain of the country. The immunity experiments carried out by the Commission, so far as they go, seem to indicate that the wild *Glossina morsitans* strain and the Nyasaland human strain are not identical; and that if they were one might expect that Sleeping Sickness would be considerably more common in Nyasaland and Rhodesia than it appears to be. On the other hand, it is conceivable that some variant of this strain may be the cause of the human disease. It does not seem possible, however, to form an opinion on this hypothesis in the absence of further information in regard to its serological and other affinities.

3. Another hypothesis, which may at any rate be applicable in some cases, was suggested by a study of the analysis of human strain III. and *Gambiense* Tanganyika Strain II. In these instances there is some evidence in favour of the strains being double infections. Is it possible that they are dual infections with, say, *T. gambiense* and a game strain? Such a suggestion might also provide an explanation for the difficulty in resolving the measurement-distributions of the human strain when the measurements are made from rats directly inoculated from human subjects. The high resistance of many animals to *T. gambiense* might result in the elimination of this factor of infection when the trypanosome was passed through animals, so that the recovered trypanosome might not be the whole infecting agent concerned in the disease in man.

When, however, the infected blood is passed directly from man to rat the *gambiense* component might persist longer and so appear in

the count, thus turning a bimodal into a multimodal distribution and rendering analysis into two components difficult or impossible. That the human strain III. and the *Gambiense* Tanganyika II. just referred to were capable of resolution may have been accidental, in that the area of the components was such that methods not ordinarily possible could be applied to them; this does not negative the suggestion that in other cases where the graph of the distribution is of a totally different shape four components may not be present. In the case of human strain X., if a dual infection occurred it might explain the difficulty in resolving the original distribution, which was obtained from a rat directly inoculated from man; whereas the two recorded successful sub-inoculations with a single trypanosome, which clearly could only represent one strain, yielded satisfactorily to mathematical analysis. It may, of course, have been merely an accident that three of the six inoculated rats failed to develop trypanosomes; on the other hand, it is possible that if two strains were present in the original case rats may be more resistant to one of them. This hypothesis might then account for some of the difficulties of the problem, but against it is the fact that the range of the two strains referred to is considerably greater than that of the other unresolvable strains. There is also no definite evidence, so far, that the components of *T. gambiense* and a game strain are sufficiently diverse in regard to the statistical constants to give rise to a distribution such as that of human strain III. Further, if future serological experiments confirm the finding that the human strain is not allied to wild *morsitans* strain and to wild game strain a further objection would be raised. Some explanation would also be required as to why the two strains persisted in the examples quoted if the general rule is that one should rapidly die out in animal passage. The fact that one example should be from Lake Tanganyika and be classed as *T. gambiense* does not seem to me necessarily a difficulty, for if these two strains exist symbiotically in Nyasaland there is no reason why they should not, given favourable circumstances, do so in other districts. The frequency of such a conjunction would depend on the chances of infection with the two strains. The fact that *palpalis* country is not *morsitans* country may account for the rarity of a mixed infection in most Sleeping Sickness districts.

This hypothesis may be considered fanciful and far-fetched and no doubt, on general principles, the simpler the hypothesis which will explain the facts the better. The chief object, however, in discussing this suggestion is to indicate the difficulties which may have to be met in solving the problem of human trypanosomiasis should a

mixed infection occur and in which one element tends to rapidly disappear when the trypanosomes are passed through animals.

Finally, it may be pointed out that unless an unmodified game strain be the cause of human trypanosomiasis—and the weight of evidence appears to me to be against this view—the human carrier is of much greater importance than the wild animal, and consequently the recommendations of the Commission cannot, unless considerably modified, be accepted as a satisfactory solution. The wholesale destruction of the big game of Africa cannot be accomplished without profoundly affecting the economic position in this Continent, nor, even if it could be satisfactorily carried out, can any positive guarantee at present be given that the situation in regard to Sleeping Sickness would be improved.

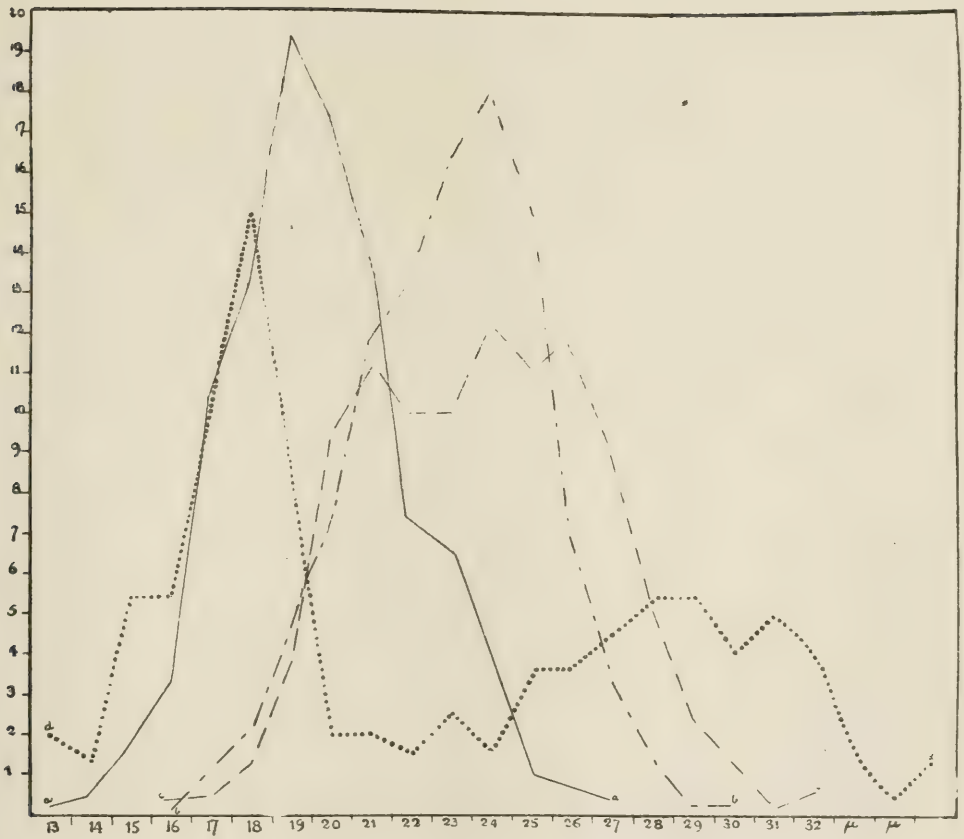
Johannesburg,
December 17th, 1915.

TABLE I.

Showing means, standard deviations, co-efficients of variability, and relative size of population of the components of trypanosome length-measurement-distributions.

	Means. 1st.	Means. 2nd.	Standard Deviation. 1st.	Standard Deviation. 2nd.	Co-efficients of Variability. 1st.	Co-efficients of Variability. 2nd.	Relative Size of Population. 1st.	Relative Size of Population. 2nd.	P.	Size of Sample.	
Human Strain I.	20·5054	27 7568	1·5867	2·6907	7·738	9·694	36·1	63 9	62	500	
.. .. II.B.	22·7695	26·8494	1·9760	1·7677	8·678	6·584	81·6	18·4	·88	500	
.. .. IV.	22·4746	26·8355	1·9708	1·9160	8·769	7·140	65·2	34·8	·85	500	
.. .. V.	22·4746	26·8355	1·9708	1·9160	8·769	7·140	65·2	34·8	·85	500	
.. .. VI.	20·2416	25·2237	1·9081	2·3032	9·427	9·131	70·8	29·2	·46	500	
.. .. VII.	19·4368	25·3298	1·1621	2·8870	5·979	11·398	47·8	52·2	·13	500	
.. .. IX.	18·3246	25·8615	1·5327	3·0992	8·364	11·984	61·8	38·2	·36	500	
.. .. X.A.	21·3203	25·4616	1·7943	1·5147	8·416	5·949	78·6	21·4	·53	500	
.. .. X.B.	20·6929	25·1799	1·3818	2·2426	6·678	8·906	31·6	68 4	·99	500	
MEAN H. STRAINS	20·9156	26·1482	1 6981	2·2597	8·091	8·658					
T. rhodesiense	18·7418	26·1122	2·3184	3·4397	12·370	13·173	34·4	65·6	·96	1,000	K.P.
.. ..	20·0552	25·4218	2·1717	2·7922	10·825	10·983	24·3	75·7	·54	1,000	
T. gambiense	19·8926	26·2463	2·0566	2·6260	10·339	10·005	65·1	34·9	·80	1,000	K.P.
.. ..	21·3545	26·3636	2·3028	2·7115	10·784	10·285	29·9	70·1	·48	1,000	
Wild Game Strain	20·4418	25·8263	1·6332	2·8799	7·990	11·151	59·5	40·5	·81	2,500	K.P.
Mzimba Strain	19·8966	24·0508	1·3961	3·1028	7·017	12·901	63·5	36·5	·26	1,000	K.P.
Liwonde Strain XXII.	19·1780	23·316	0·7619	2·0849	3·972	8·942	8·0	92·0	·95	500	
T. brucei XXV., 2nd pass. ...	18·8879		1·4839		7·856		35·4		·88	500	
	20·2092		2·4728		12·236		64·6				
.. Z. Strain, 1896 ...	17·6435	28·6851	2·0002	2·7898	11·337	9·726	56·0	44·0	·44	200	
.. XXV., 1st pass. ...	20·4358	25·6839	1·6854	2·5518	8·247	9·935	63 2	36·8	·015	500	
GRAND MEAN	20·2489	25·9467	1·7783	2·5175	8·7894	9·890					
T. brucei XXV., 2nd pass. ...	19·4270	23·3668	2·0186	1·9566	10·391	8·373	92·4	7·6	·75	500	
Human Strain III.	17·8831	24·5319	0·7184	2·0129	4·017	8·205	12·0	25·8	·25	1,000	
	19·6010	29·7892	0·7651	2·6183	3·903	8·789	5·4	56·0			
T. gambiense, Tang. II.... ...	18·4882	26·7097	1·3448	1·3442	7·274	5·033	24·0	19·4	·99	500	
	23·1988	30·1355	1·5955	2·0357	6·878	6·755	12·8	44·6			

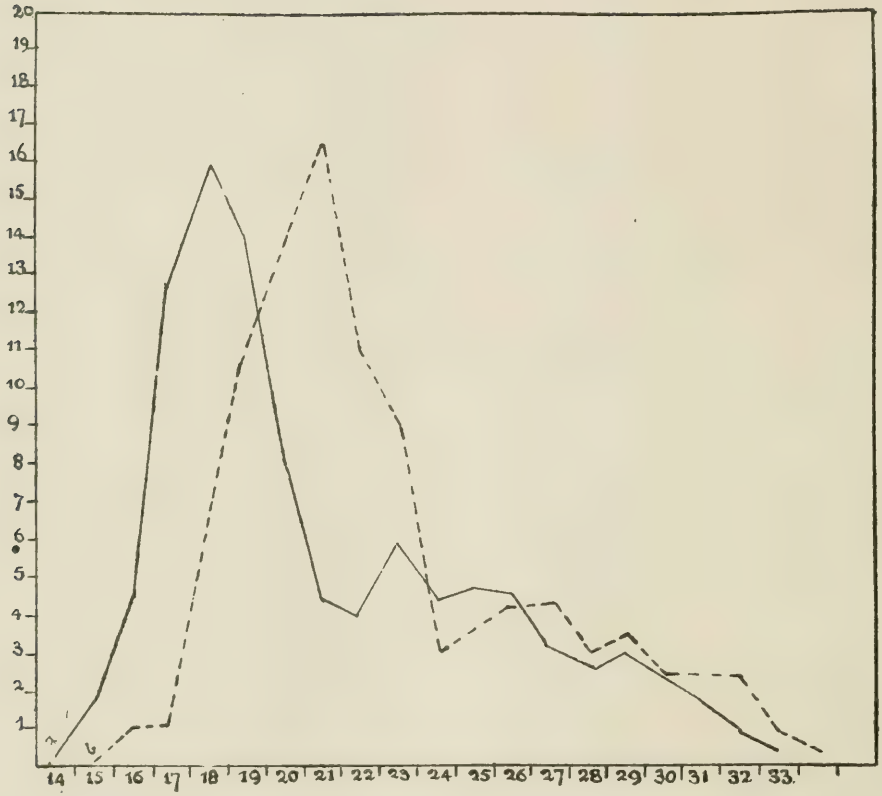
CHART I.



SLEEPING SICKNESS COMMISSION. Royal Society's Report.

a ———— *Brucei.* Chart 21, p. 81.
b — - - - — *Liwonde.* „ 10, p. 61.
c — — — - - *Human N.* „ 8, p. 56.
d ..... *Brucei.* „ 16, p. 74.

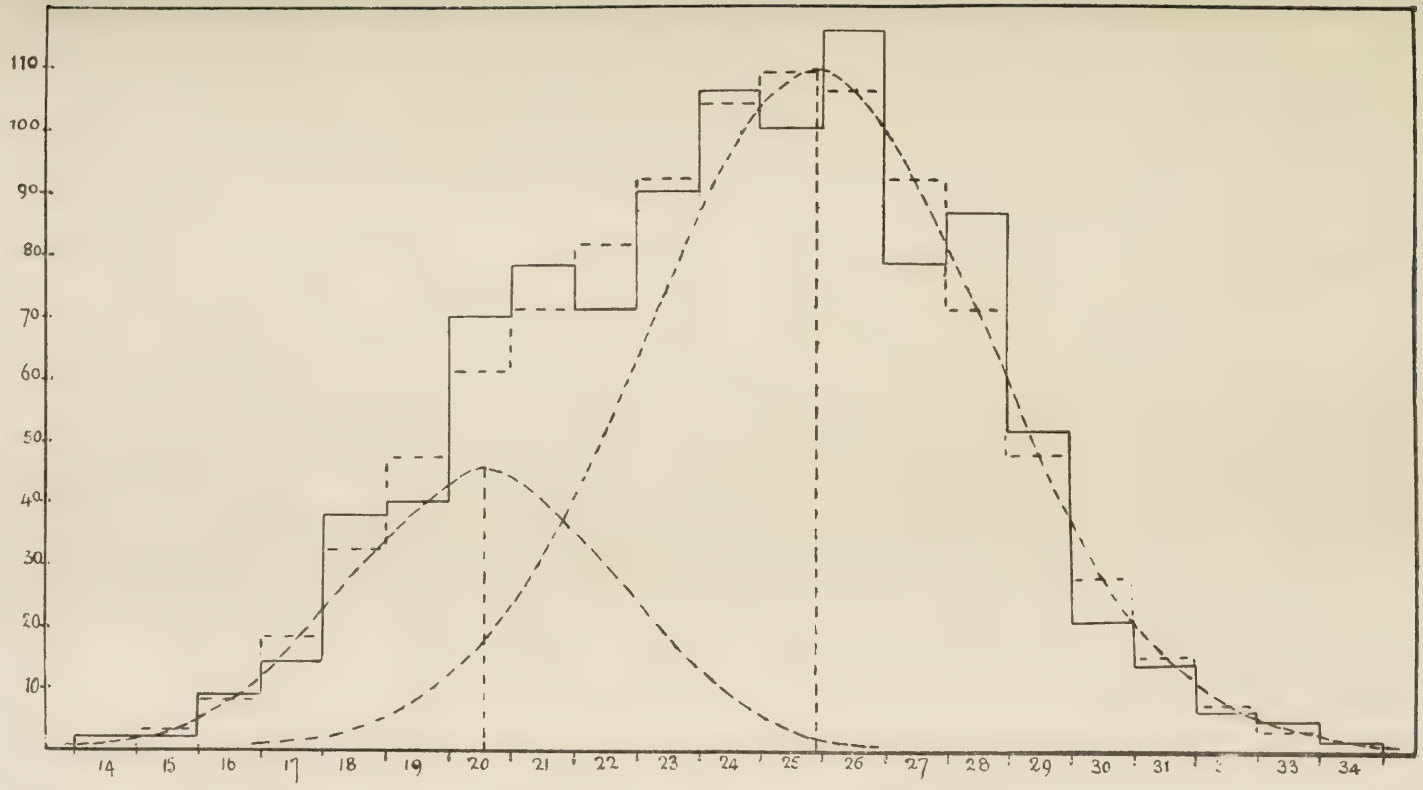
CHART II.



Sleeping Sickness Commission. Royal Society's Report.

a ——— *Human Strain LV.* Chart 4. p. 51.

b — — — *Gambiense.* Chart 33. p. 190.

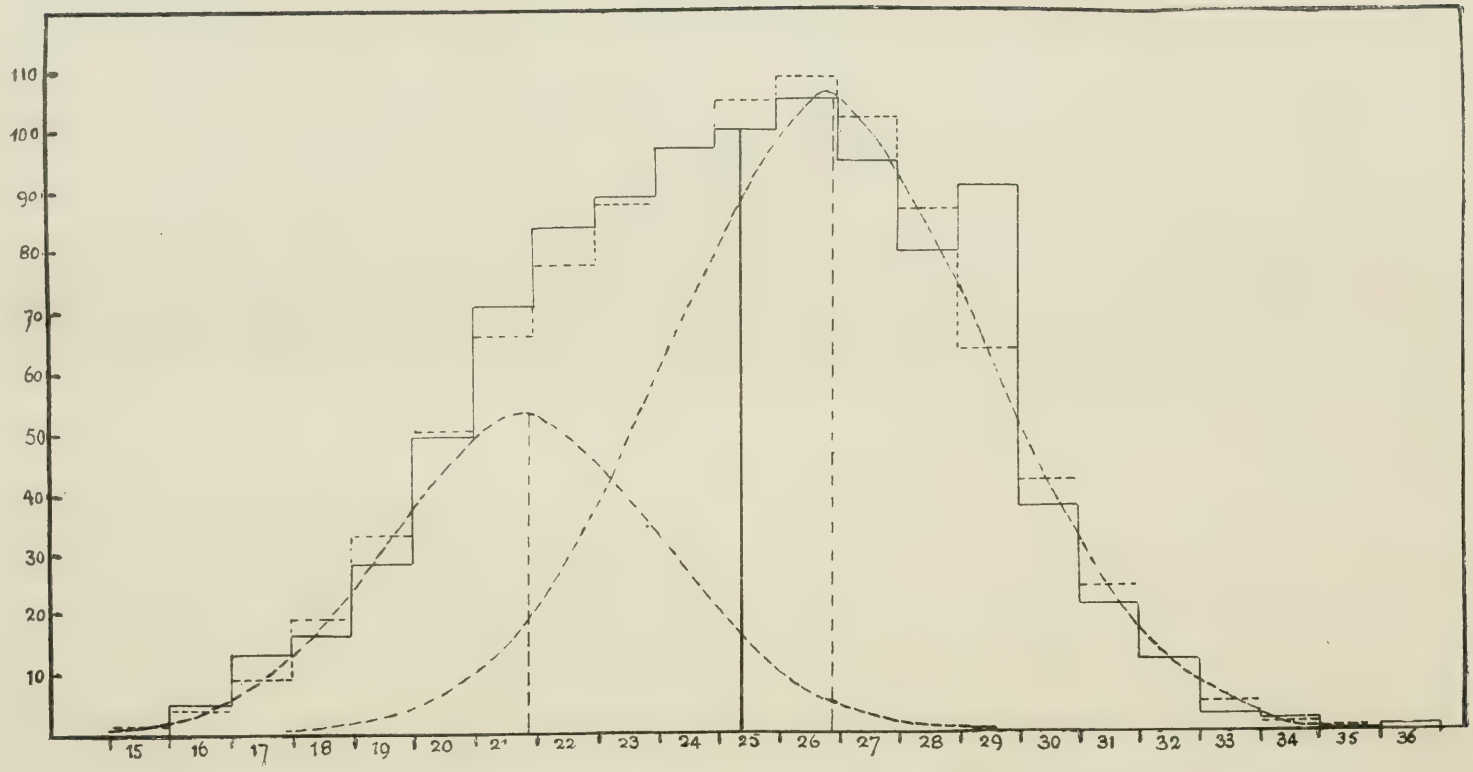


"Annals of Trop. Med. & Par." Vol. VII. p. 28.

Trypanosoma rhodesiense.

	Mean.	σ	N.
Obs.	24·121		1,000
I.	20·0552	2·1717	243
II.	25·4218	2·7922	757

$\chi^2 = 18{\cdot}74$. $P = {\cdot}54$.

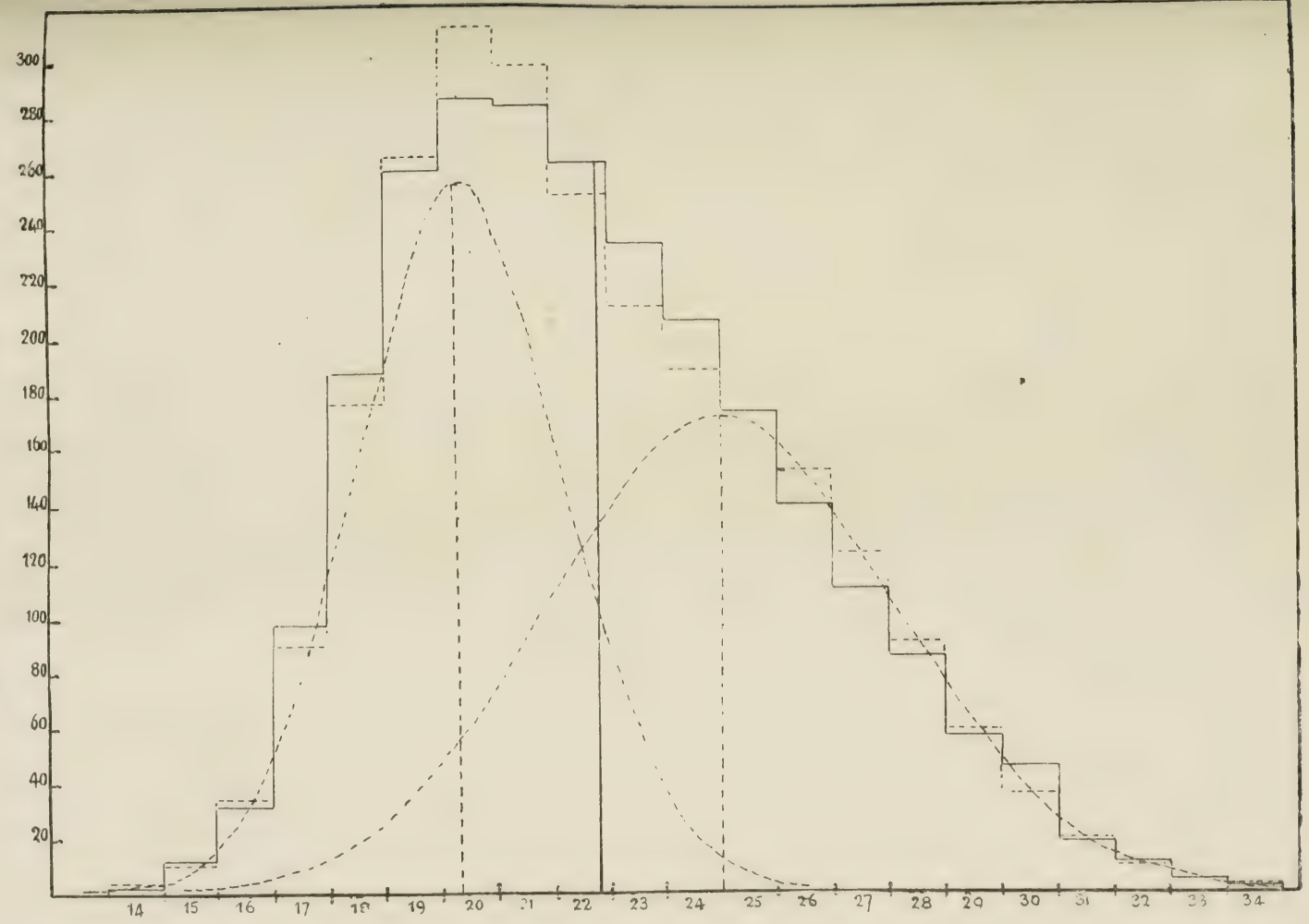


"Annals of Tropical Med. & Par." Vol. VII. p. 32.

Trypanosoma gambiense.

	Mean.	σ	N.
Obs.	24·867	—	1,000
II.	21·3545	2·3082	299
III.	26·3636	2·7115	701

$\chi^2 = 18{\cdot}64$. $P = {\cdot}48$.



	Mean.	σ	N.
Obs.	22·2872	3·4822	2,500
I.	19·8271	1·8305	1,178
II.	24·4788	3·0877	1,322

$P_2 = -5.3916$ $P = .77$.

CHART V.

SLEEPING SICKNESS COMMISSION. Royal Society's Report.

Human Strains VI.—X. (through rat).

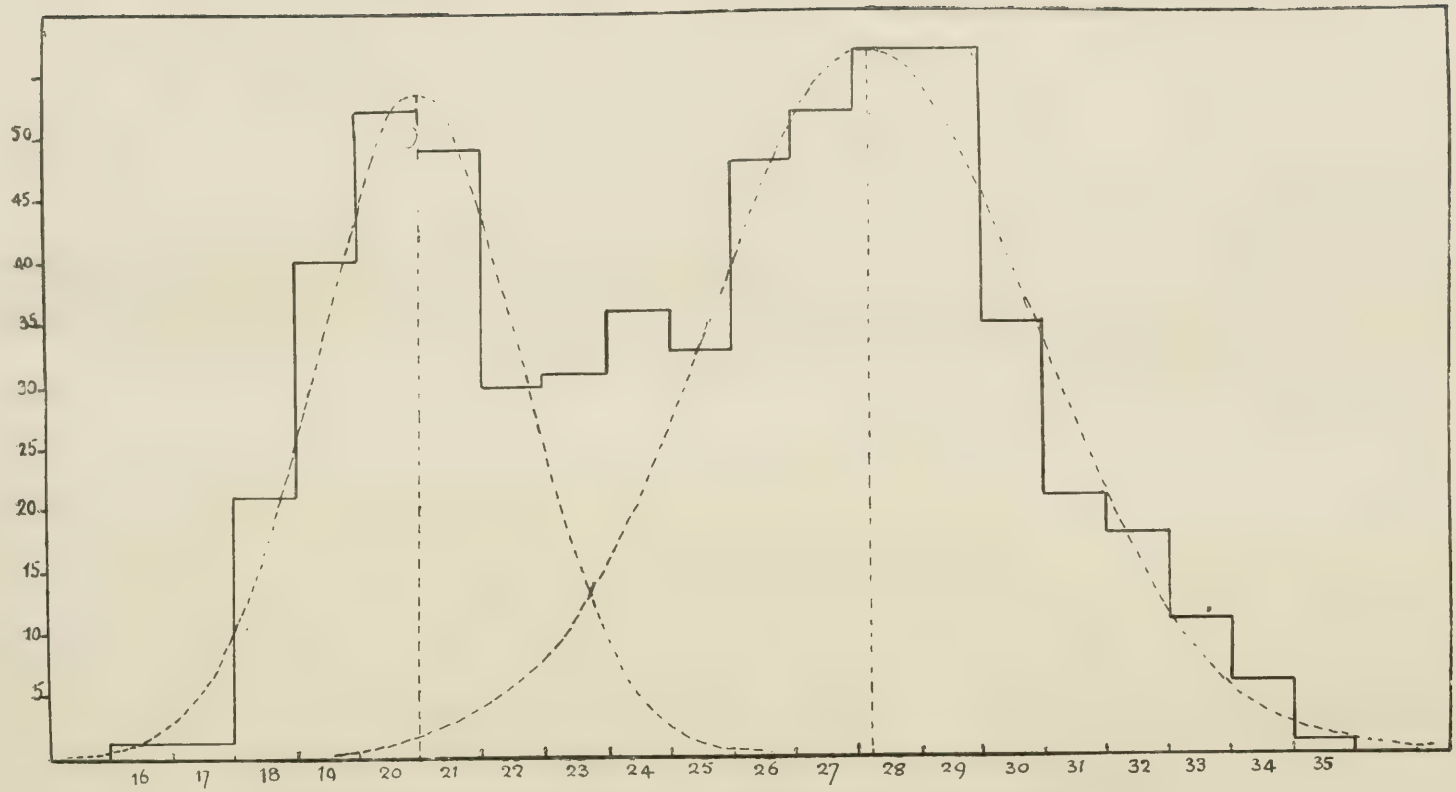


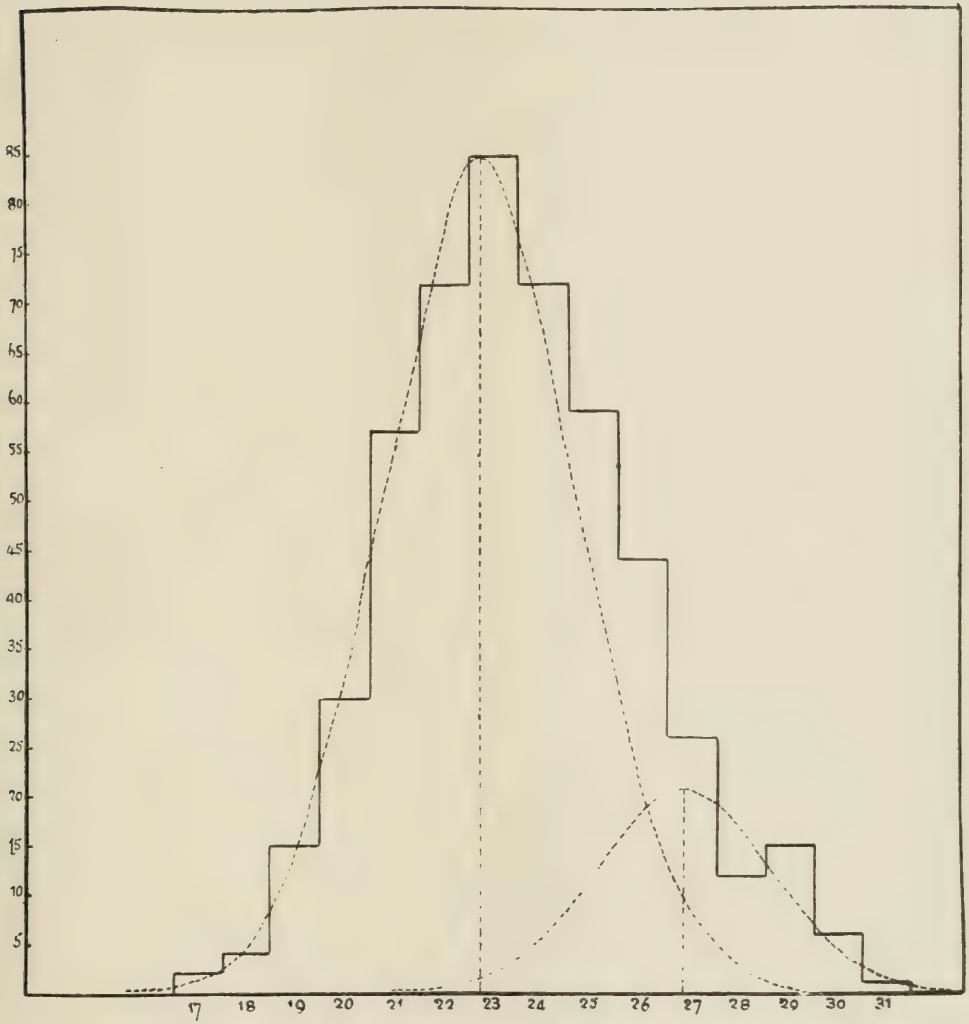
CHART VI.

SLEEPING SICKNESS COMMISSION. *Human Strain I.* (Through rat only.)

Mean.	σ	N.
20·5054	1·5867	217
27·7568	2·6907	383

$p_2 = -12{\cdot}1344$. $P = {\cdot}62$.

CHART VII.



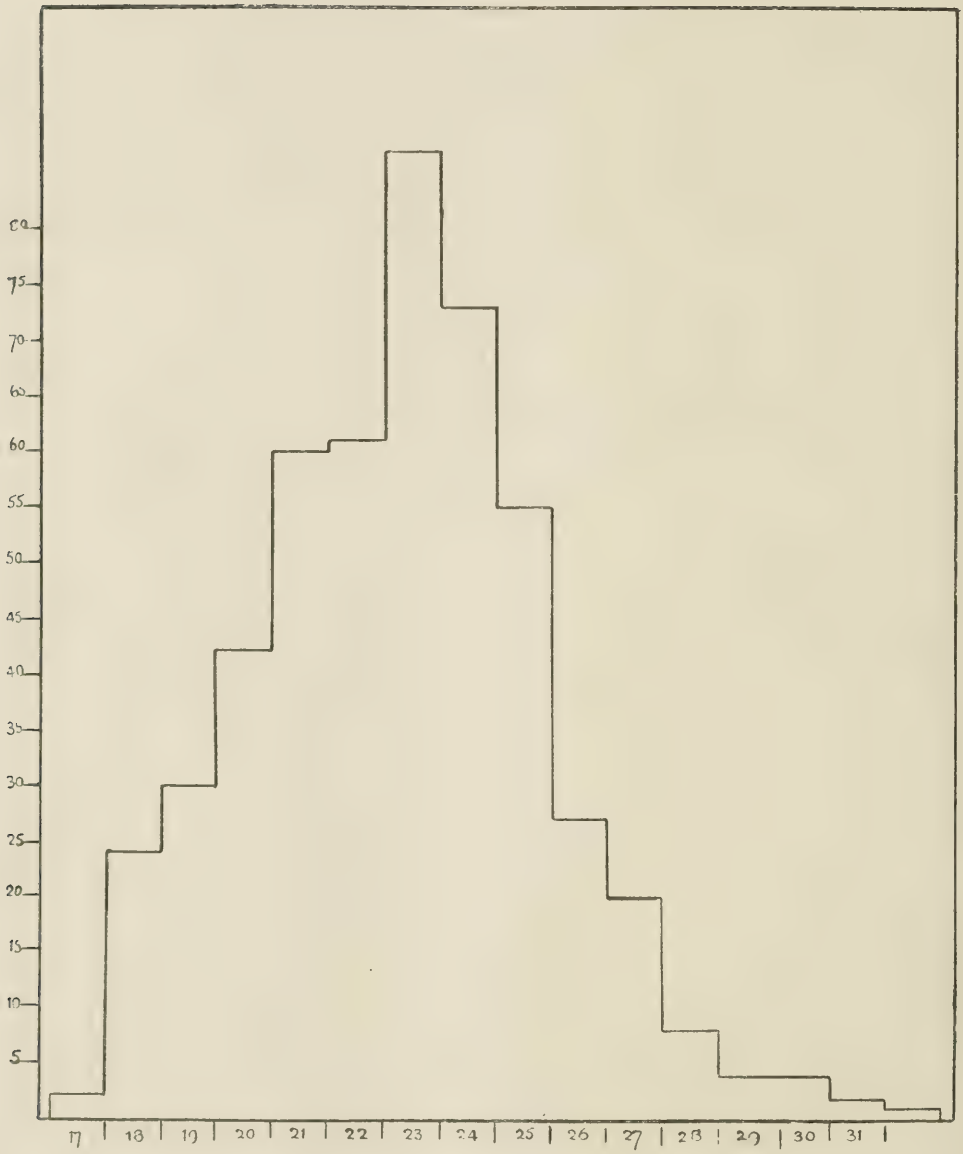
Sleeping Sickness Commission. Royal Society's Report.

Human Strain II. Rat 728.

Mean	σ	N.
22·7695	1·9760	408
26·8494	1·7677	92

$p_2 = -2{\cdot}4884.$ $P = {\cdot}88$

CHART VIII.



Sleeping Sickness Commission. Royal Society's Report.

Human Strain II.B. Rat 726.

Mean.	σ	N.
22·804	2·5981	500

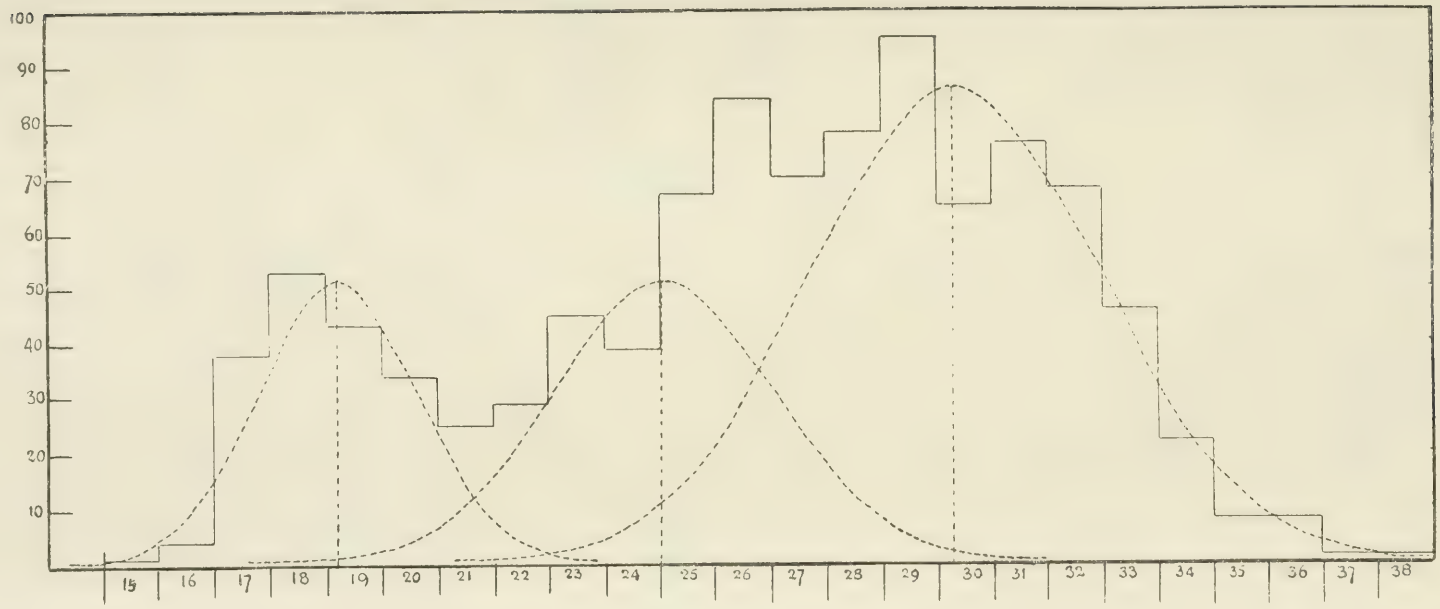


CHART IX.

SLEEPING SICKNESS COMMISSION.

Human Strain III. (Through rat only.)

Mean.	σ	N.
18·7044	1·4107	182
24·5319	2·0129	258
29·7893	2·6183	560

$P = \cdot 28$.

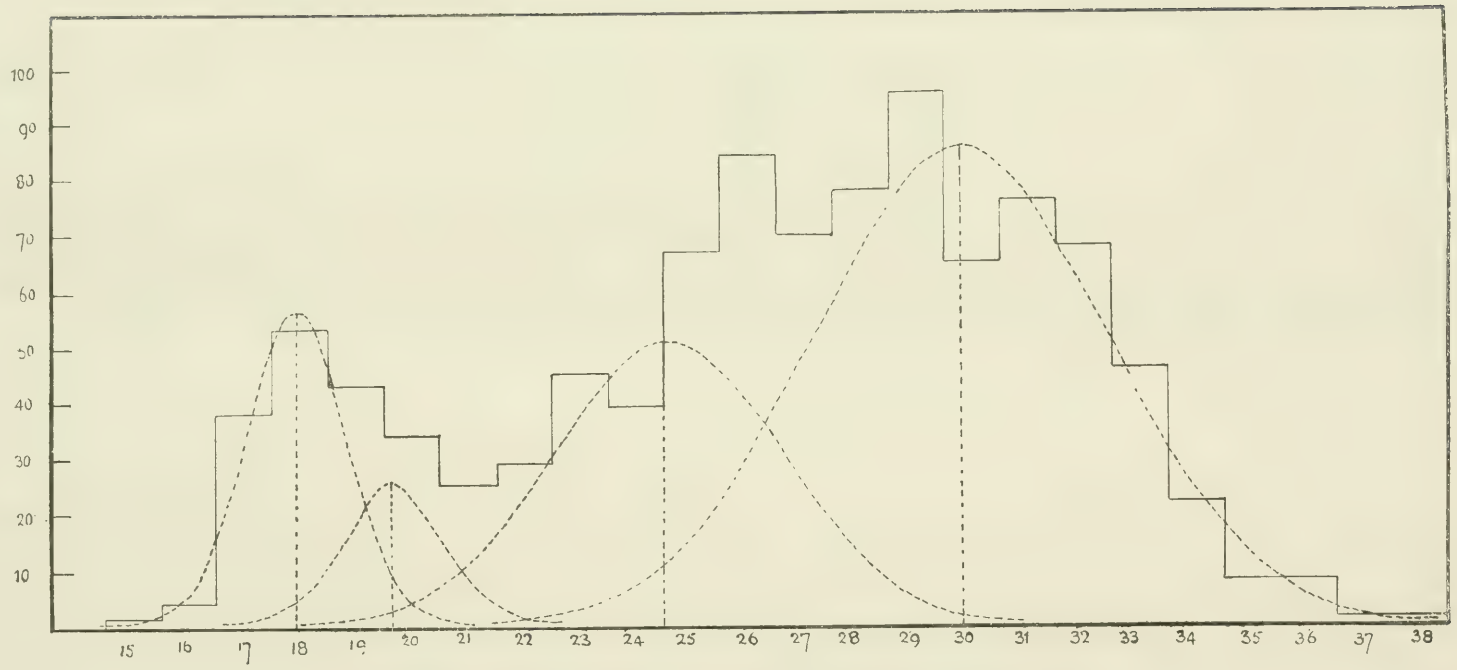


CHART X.

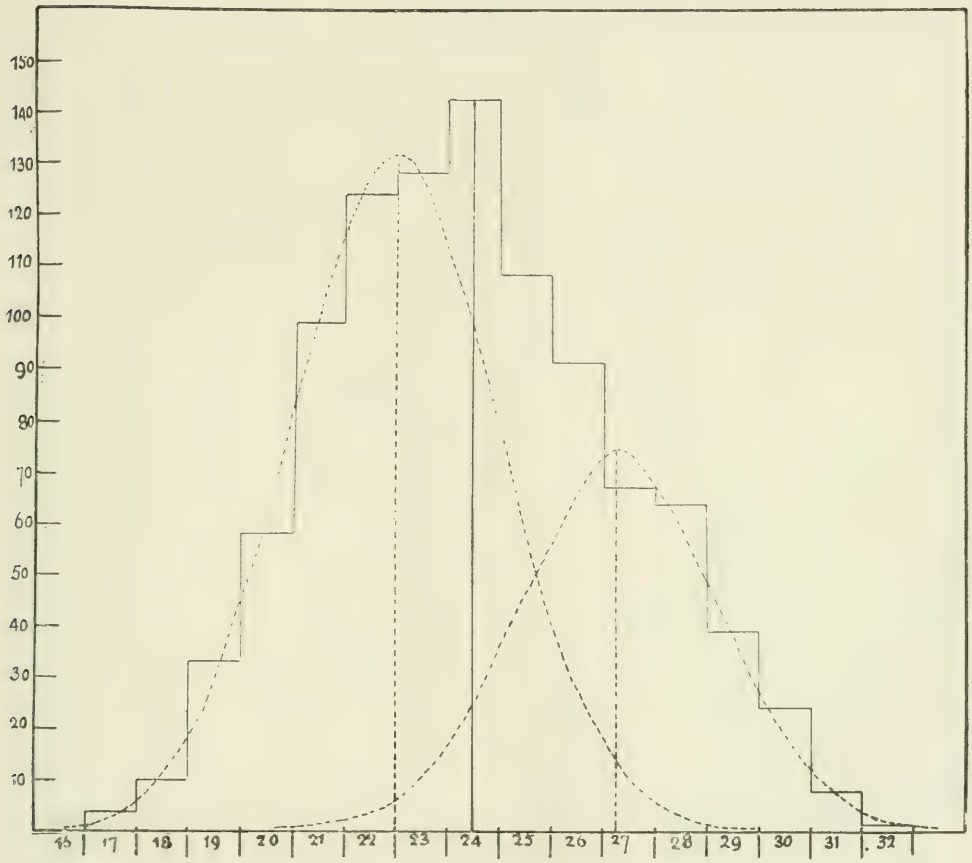
Sleeping Sickness Commission. Royal Society's Report.

Human Strain III., Rat.

Mean.	σ	N.
17·8831	0·7184	120
19·6010	0·7651	54
24·5319	2·0129	258
29·7893	2·6183	560

$P = \cdot 25$.

CHART XI.



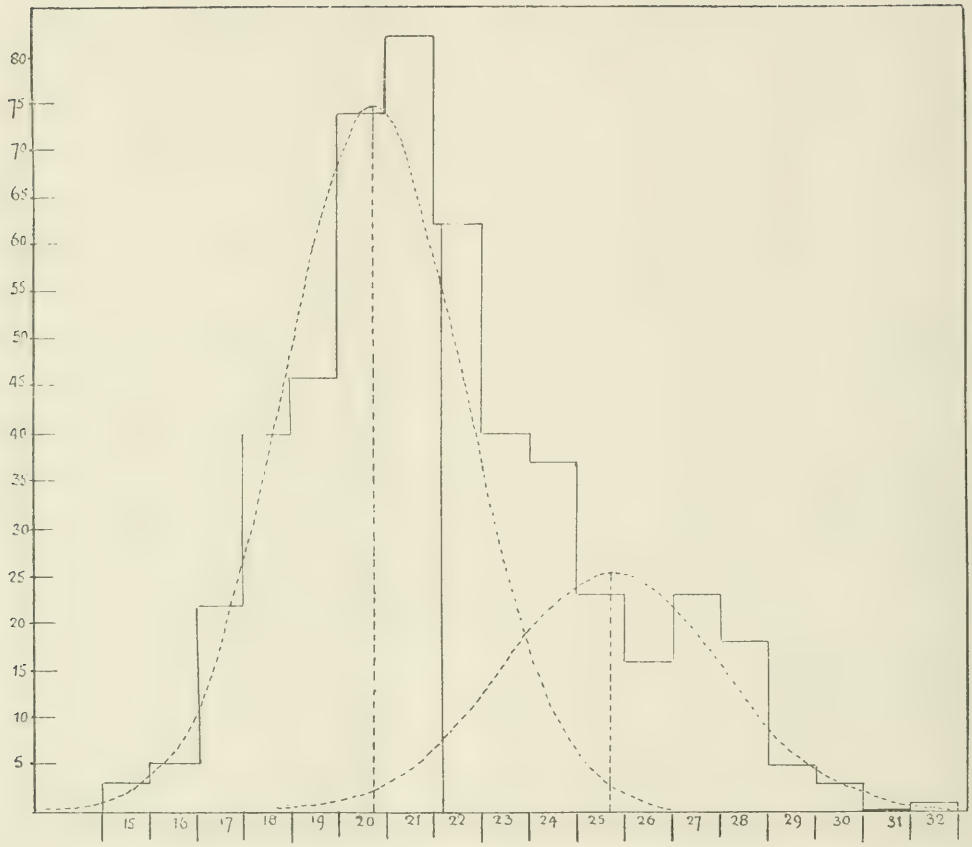
Sleeping Sickness Commission.

Human Strains IV. and V. Through rat only.

Mean.	σ	N.
22·4746	1·9708	652
26·8355	1·9160	348

$P = \cdot 85$. $\beta_1 = 0 \cdot 048332$. $\beta_2 = 2 \cdot 520952$.

CHART XII.



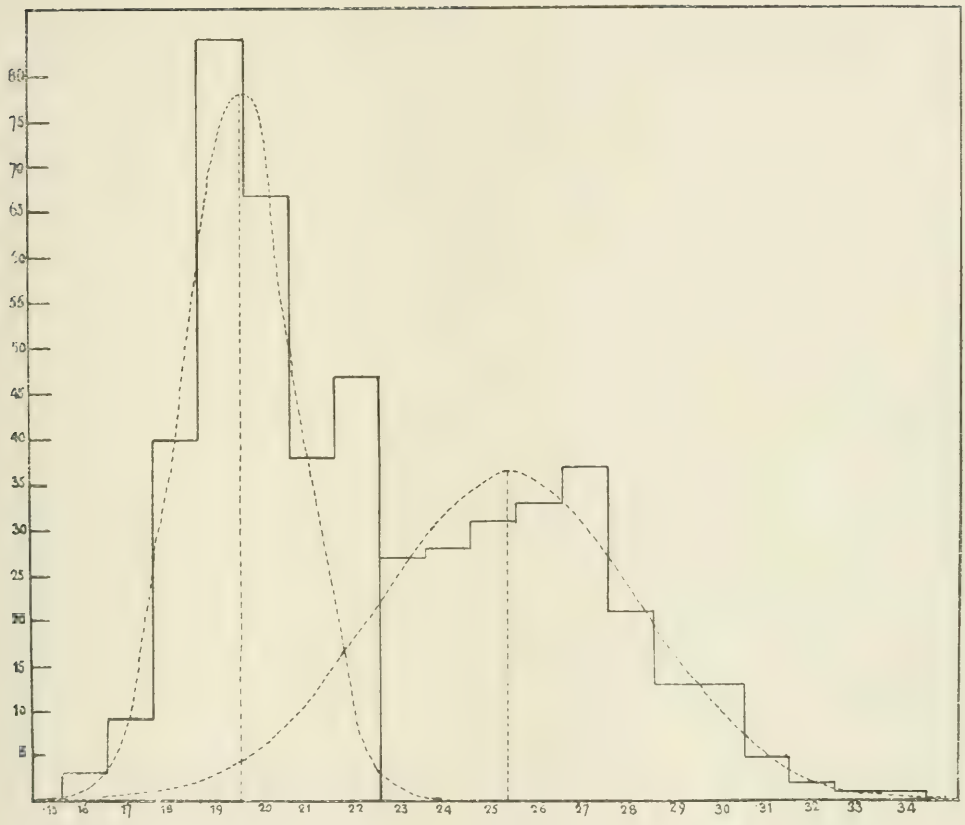
Sleeping Sickness Commission. Royal Society's Report.

Human Strain VI. Rat only.

Mean.	σ	N.
21·7000		
20·2416	1·9081	354
25·2237	2·3032	146

$p_2 = -5{\cdot}1390.$ $P = {\cdot}46.$

CHART XIII.



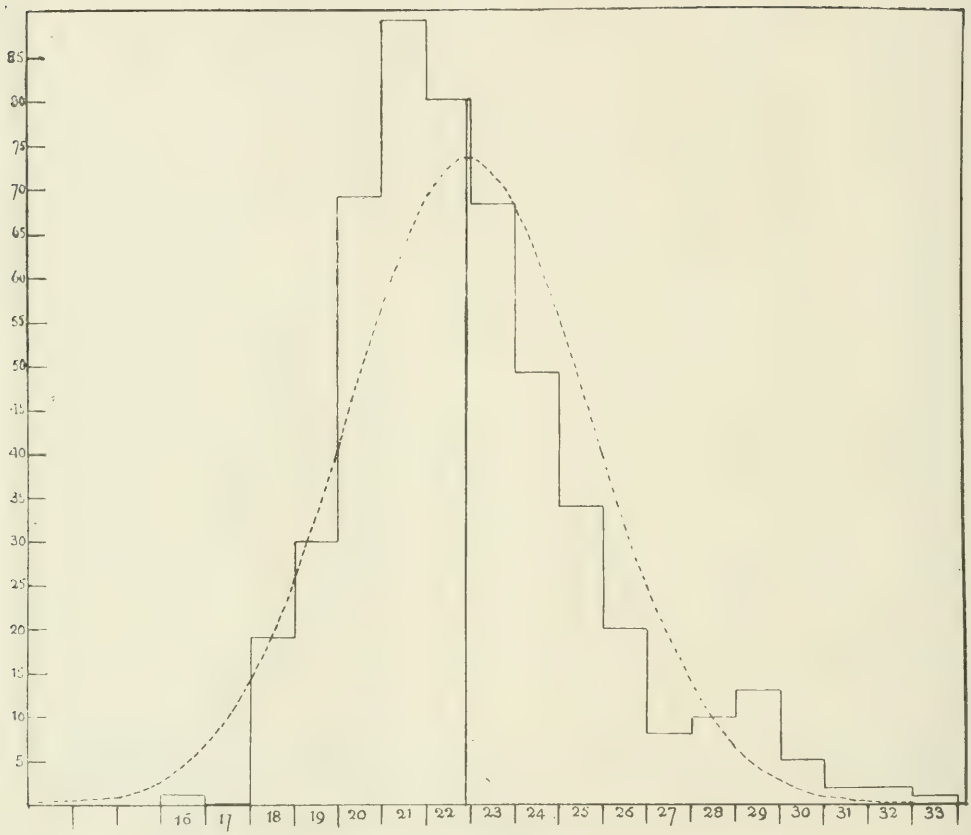
Sleeping Sickness Commission. Royal Society's Report.

Human Strain VII. Through rat only.

Mean.	σ	N.
19·4368	1·1621	239
25·3298	2·8870	261

$p_2 = -8{\cdot}6657$ $P = {\cdot}13$. Excluding χ^2 for Group 22 $P = {\cdot}93$.

CHART XIV.



Sleeping Sickness Commission. Royal Society's Report.

Human Strain VIII., from rat.

Mean.	σ	N.
22·458	2·7271	500

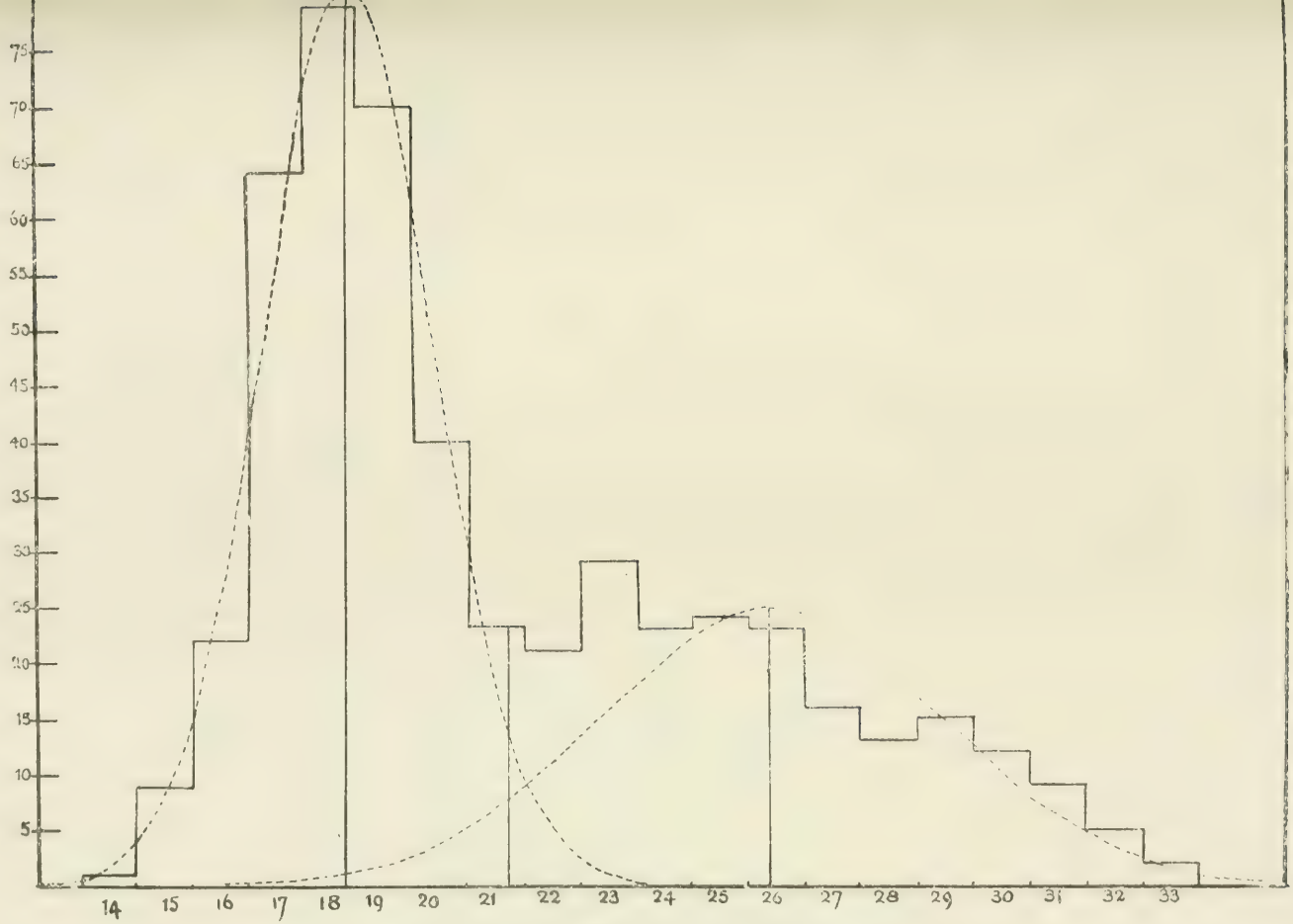


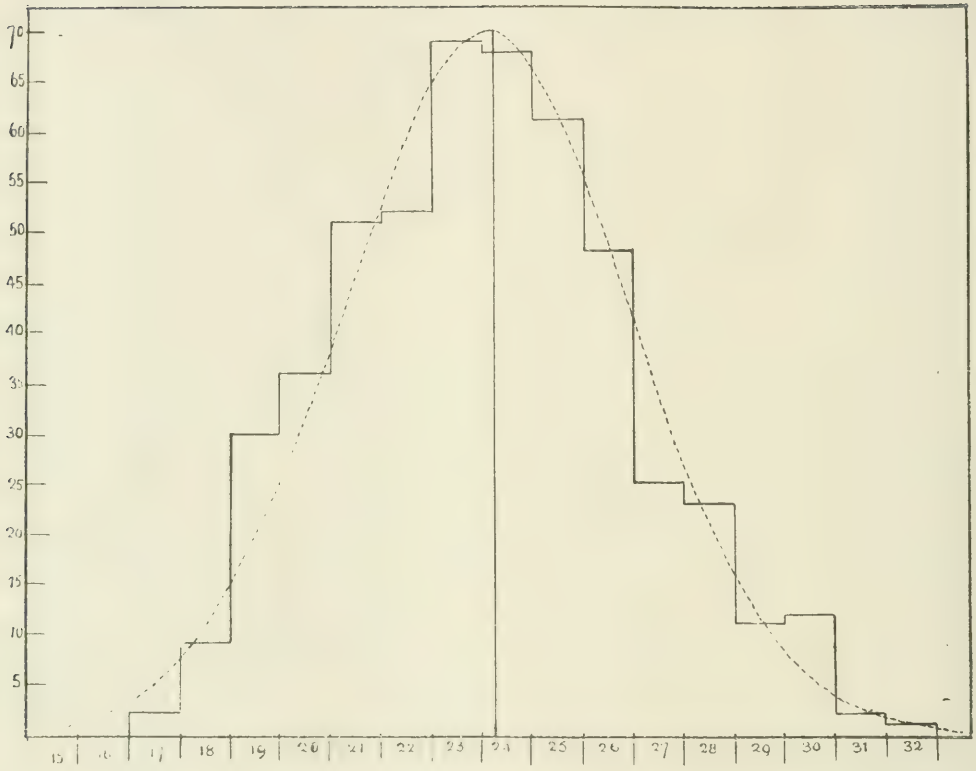
CHART XV.

SLEEPING SICKNESS COMMISSION.

Human Strain IX. Through rat only.

Mean.	σ	N.
18·3246	1·5327	309
25·8615	3·0992	191

CHART XVI.



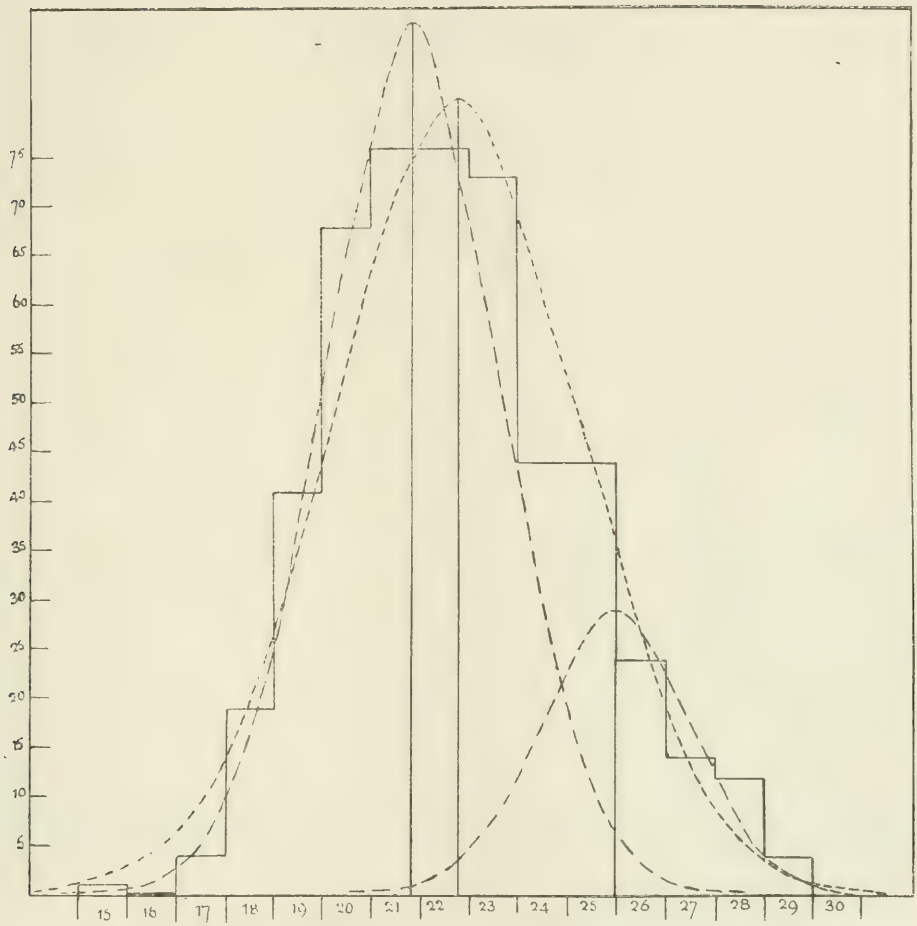
Sleeping Sickness Commission. Royal Society's Report.

Human Strain N., through rat.

Mean.	σ	N.
23·5709	2·8513	500

$\beta_1 = 0{\cdot}0483.$ $\beta_2 = 2{\cdot}6418.$ $P = {\cdot}156$

CHART XVII.



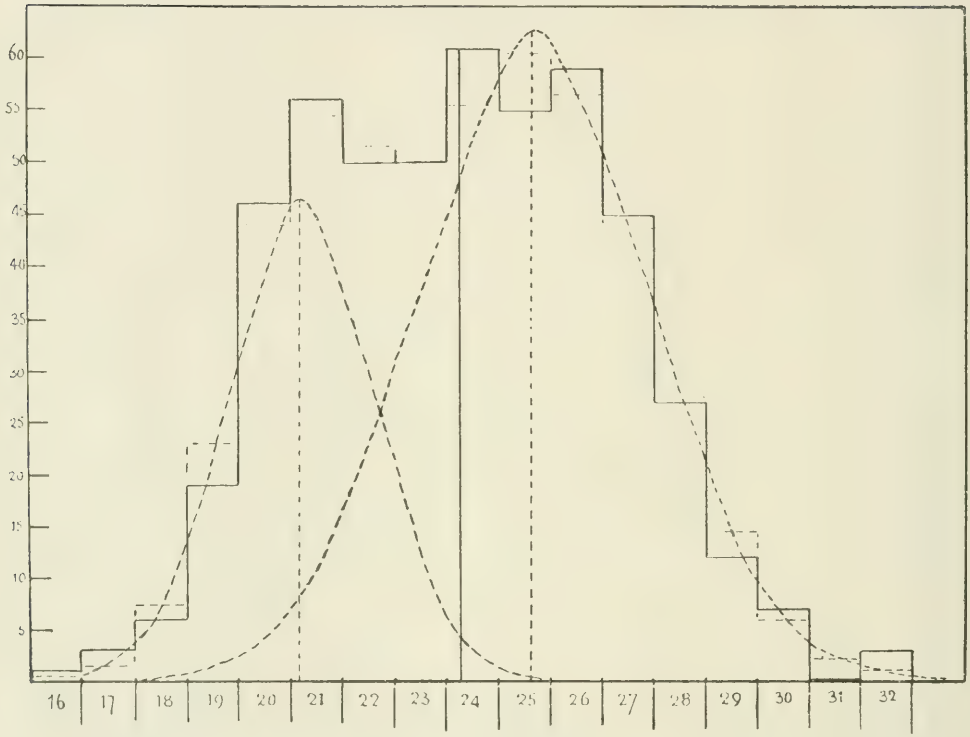
Sleeping Sickness Commission. Royal Society's Report.

Human Strain X.A. Rat—one infecting trypanosome.

Mean.	σ	N.	
22·242	2·4723	500	
21·3203	1·7943	343	$P = ·025$.
25·4616	1·5147	107	

$p_2 = -3·1014$. $P = ·53$.

CHART XVIII.



Sleeping Sickness Commission. Royal Society's Report.

Human Strain X.B. Rat—one infecting trypanosome.

	Mean.	σ	N.
Obs.	23·7600	—	500
	20·6929	1·3818	158
	25·1799	2·2426	342

$p_2 = -4{\cdot}3550.$ $P = {\cdot}99.$

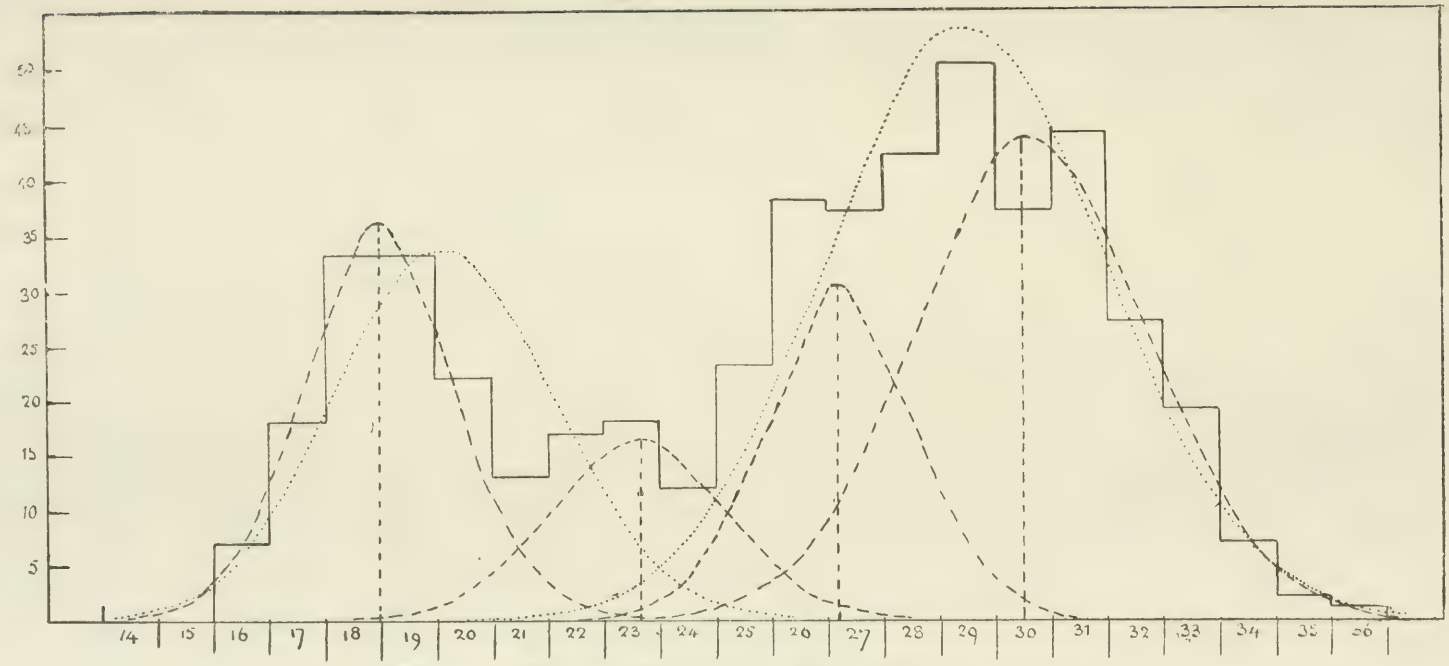


CHART XIX.

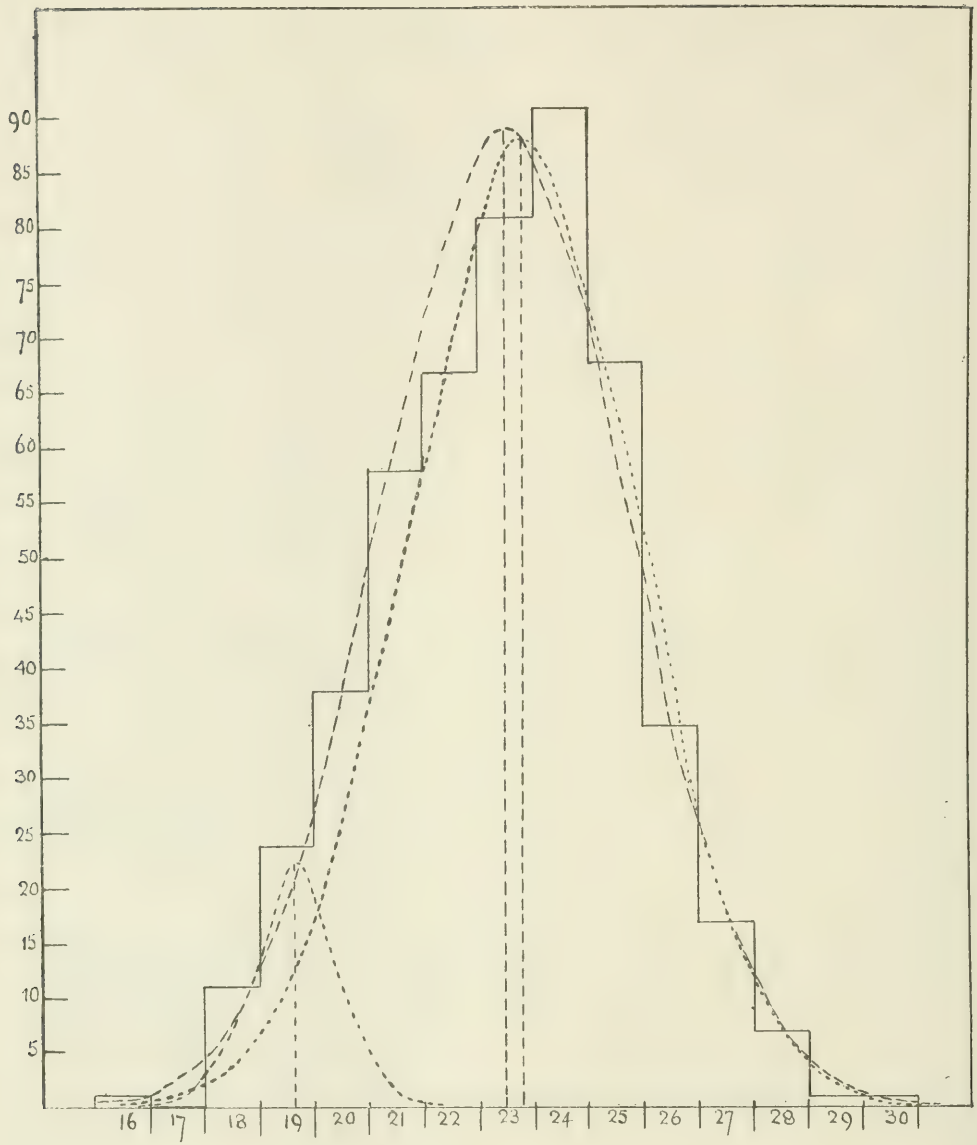
Sleeping Sickness Commission. Royal Society's Report.

T. gambiense, Tanganyika Strain II. Rat.

Mean.	σ	N.	
19·6053	1·9398	166	$p_2 = -19{\cdot}4366$.
28·9625	2·4919	334	$P = {\cdot}01$.
18·4882	1·3448	120	$p_2 = -4{\cdot}8305$.
23·1988	1·5955	64	$P = {\cdot}82$.
26·7097	1·3442	97	+28·96.
30·1355	2·0357	223	

$P = {\cdot}99$.

CHART XX.

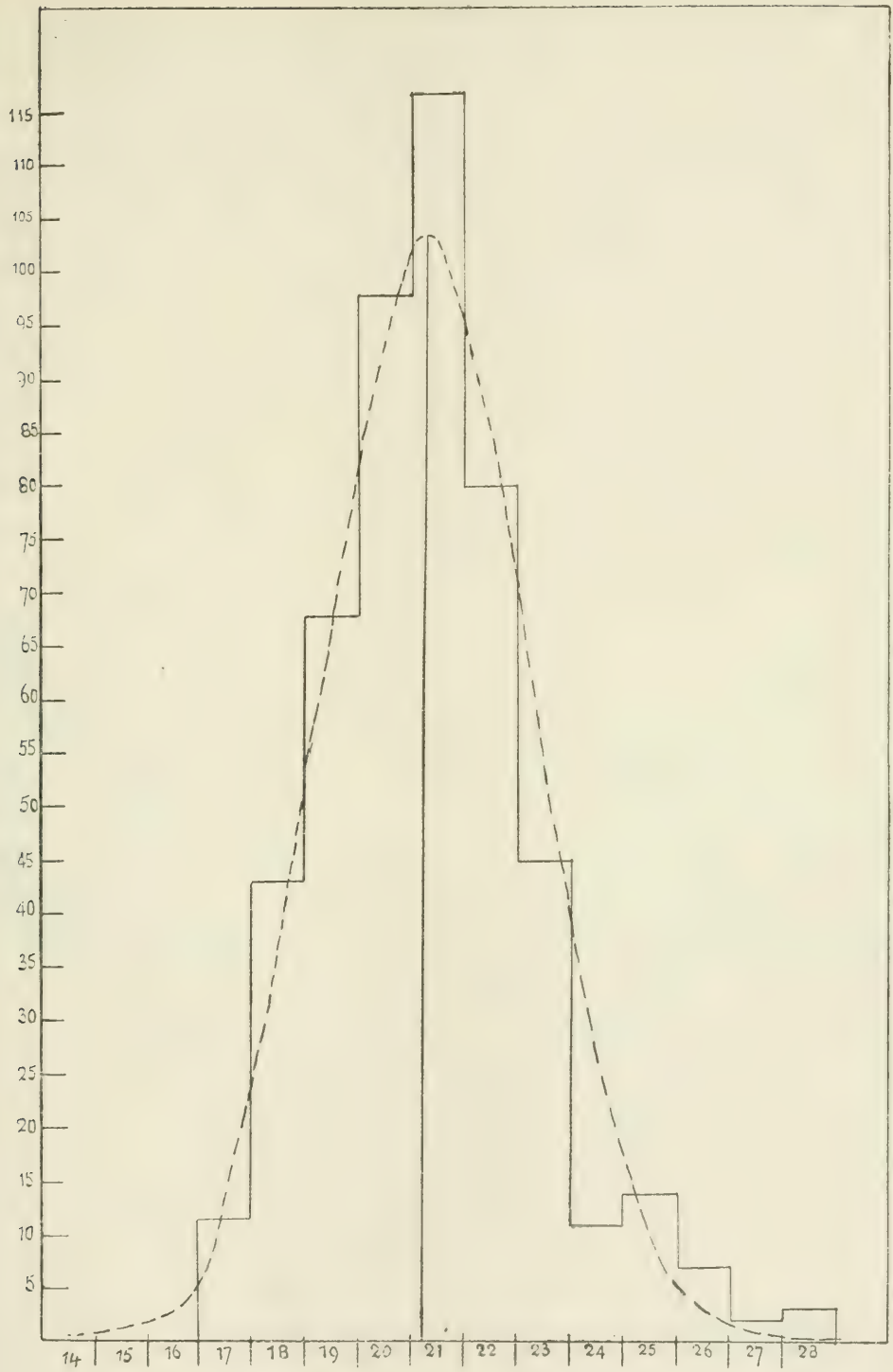


Sleeping Sickness Commission. Royal Society's Report.

Liwonde Strain XXII. (p. 61.)

Mean.	σ	N.
22·986	2·2438	500
		$P = ·62$
19·178	0·7619	40
23·316	2·0849	460

$p_2 = -·2554.$ $P = ·95.$

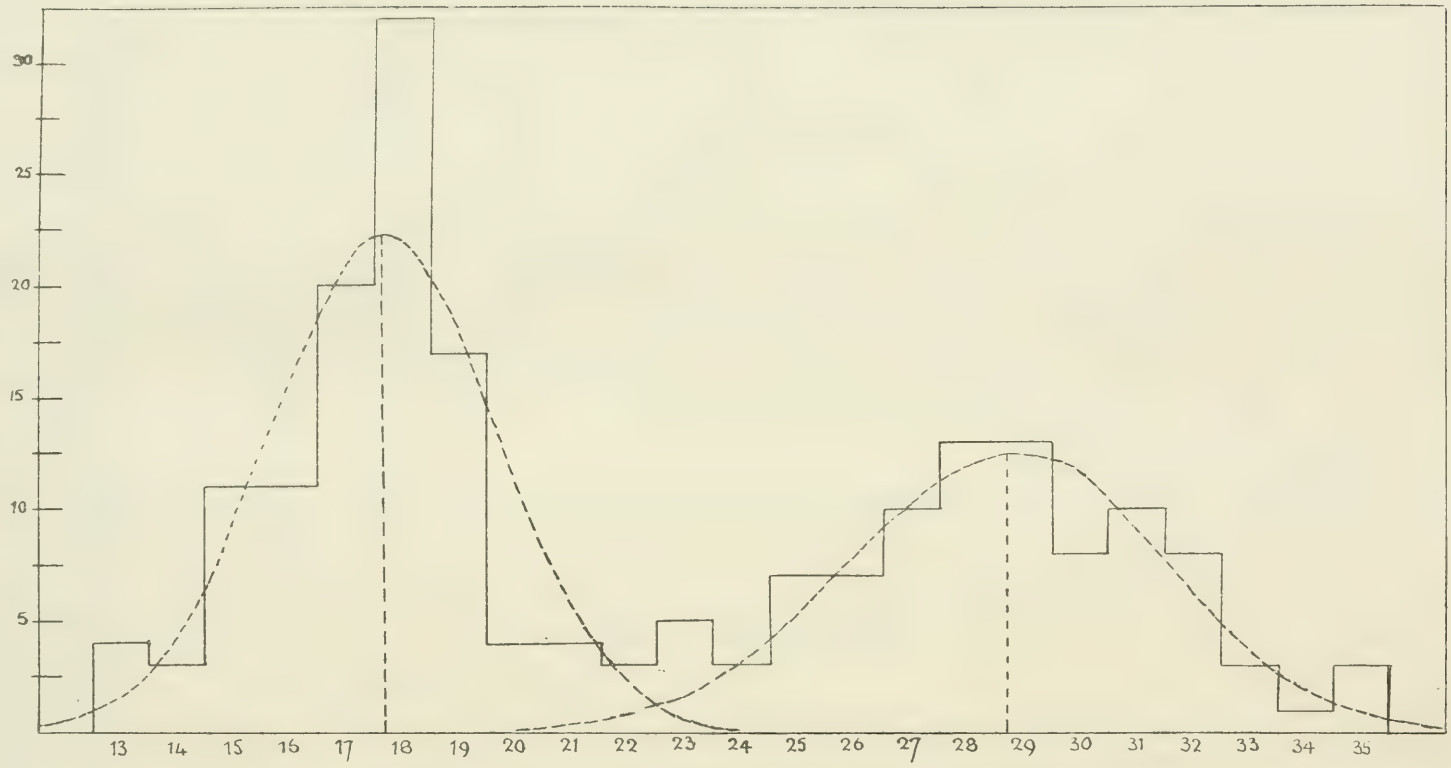


SLEEPING SICKNESS COMMISSION. Royal Society's Report.

T. brucei, Zululand Strain XXV. (p. 80.)

Mean.	σ	N.
20·832	1·9340	500

$\beta_1 = 0{\cdot}4271.$ $\beta_2 = 3{\cdot}9325.$ $P = {\cdot}002.$



Sleeping Sickness Commission. Royal Society's Report.

T. brucei—Zululand Strain 1896.

Mean.	σ	N.
17·6435	2·0002	112
28·6851	2·7898	88

$p_2 = -30{\cdot}5770.$ $P = {\cdot}44.$

CHART XXIII.



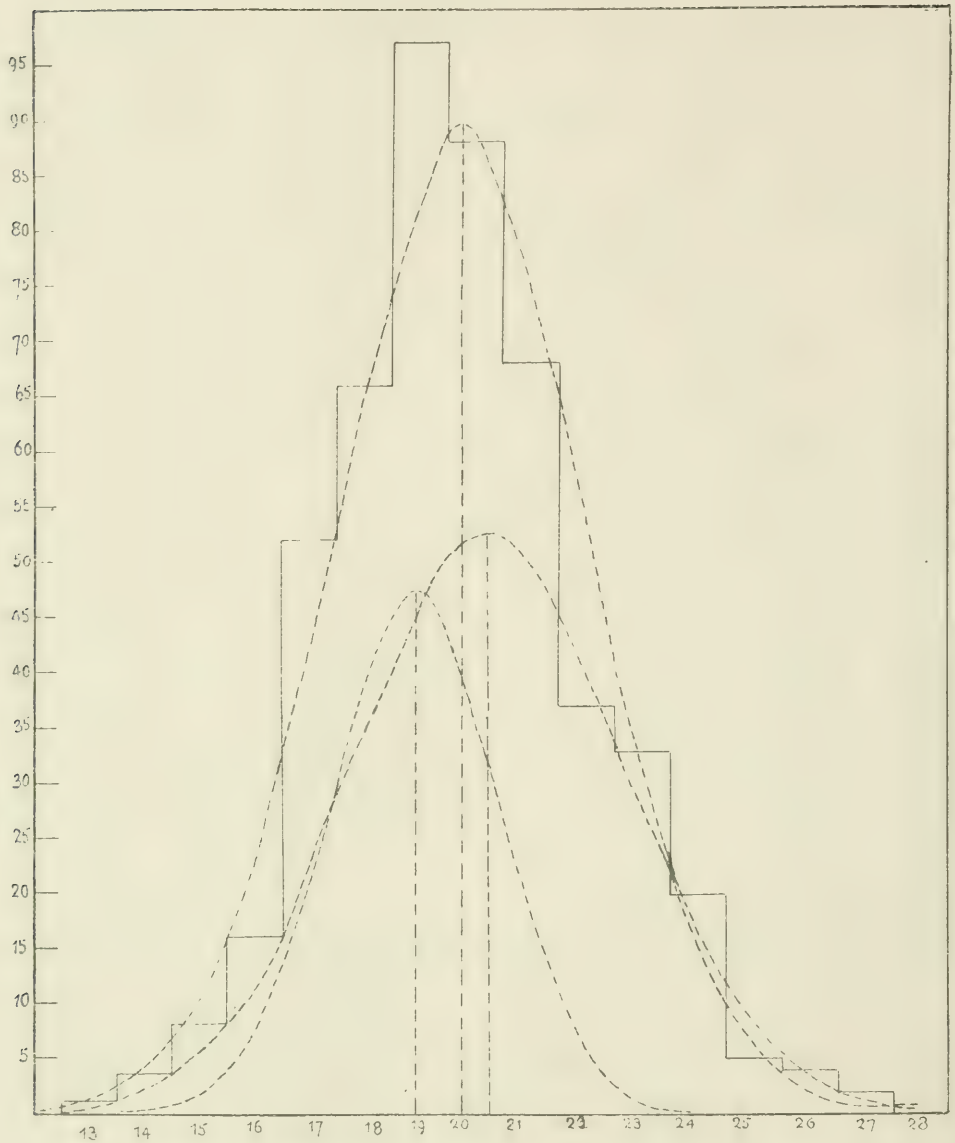
Sleeping Sickness Commission. Royal Society's Report.

T. brucei XXV. First passage through *G. morsitans*.

Mean.	σ	N.
20·4358	1·6854	316
25·6839	2·5518	184

$p_2 = -6{\cdot}4042$. $P = {\cdot}015$.

CHART XXIV.



SLEEPING SICKNESS COMMISSION. Royal Society's Report.

Brucei Strain XXV., after 2nd passage through *Glossina morsitans*.
(p. 81.)

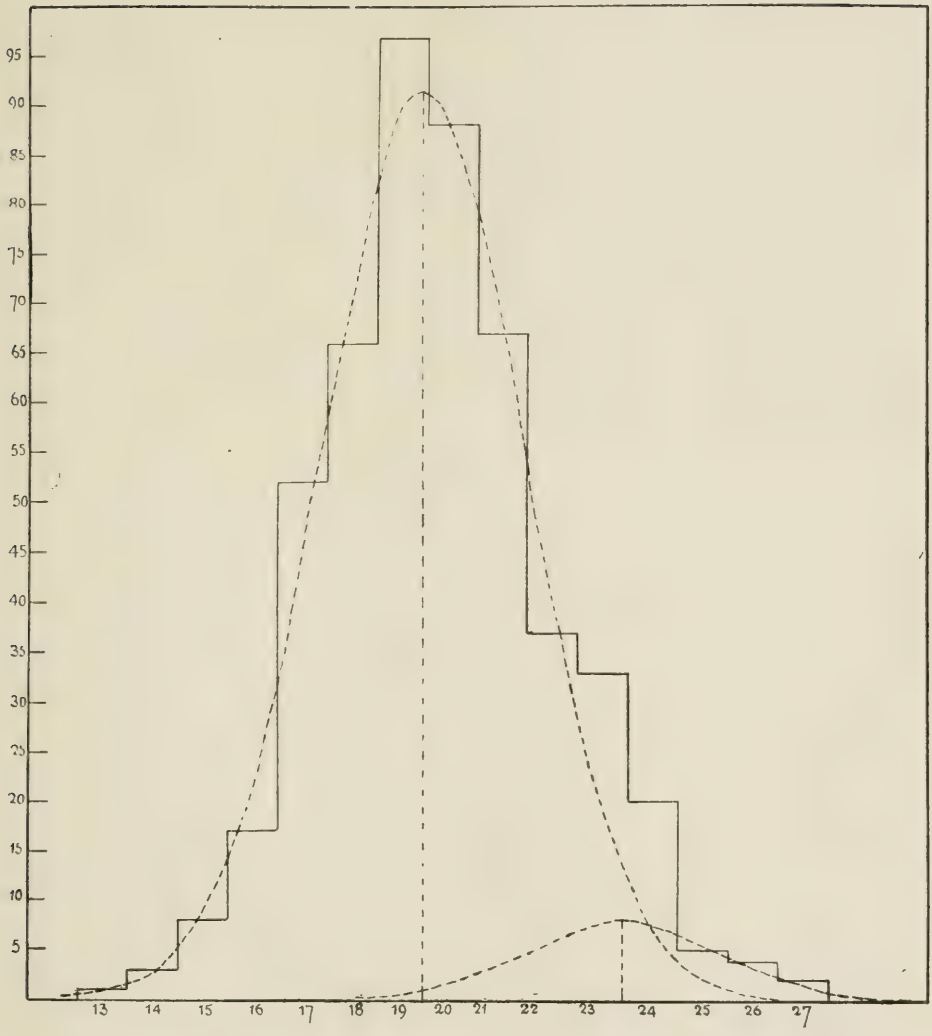
Mean.	σ	N.
19·742	2·2650	500

$\beta_1 = 0{\cdot}0851.$ $\beta_2 = 3{\cdot}1710.$ $P = {\cdot}19.$

Mean.	σ	N.
18·8879	1·4839	177
20·2092	2·4728	323

$p_2 = -0{\cdot}3990.$ $P = {\cdot}88.$

CHART XXV.



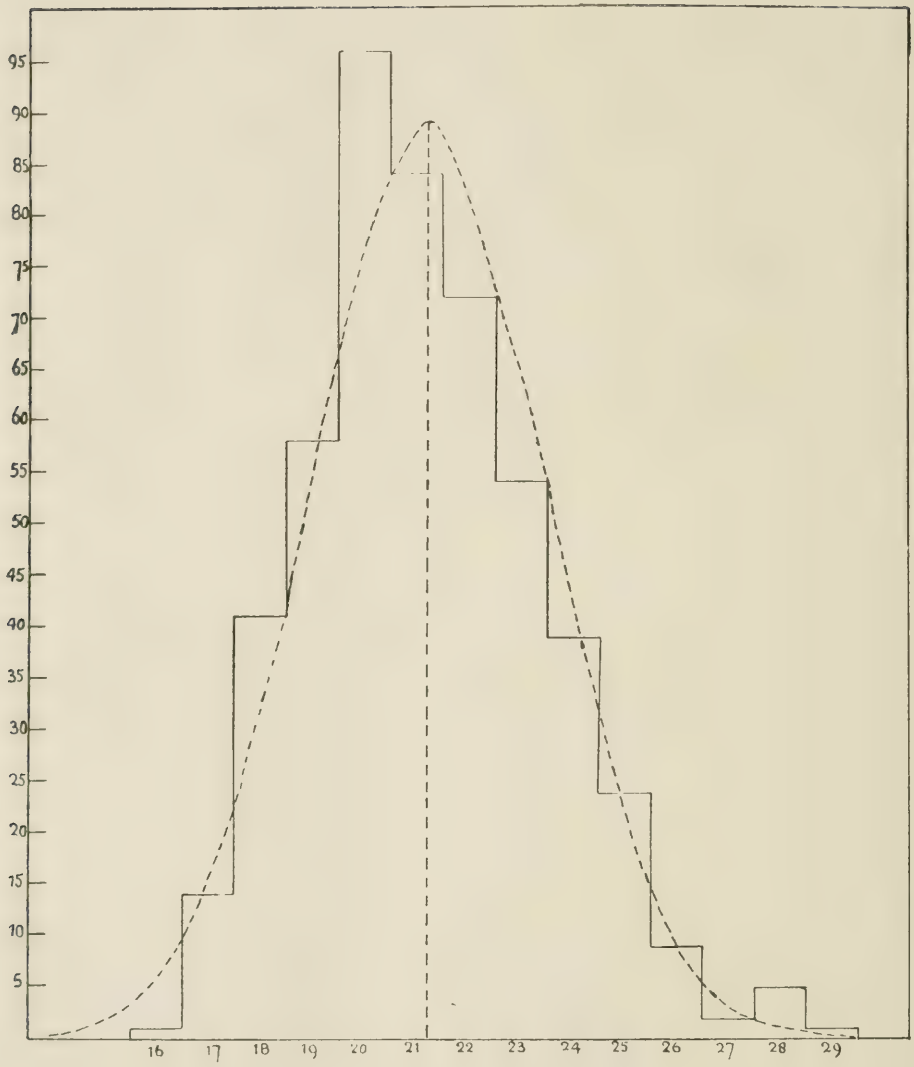
Sleeping Sickness Commission. Royal Society's Report.

T. brucei XXV. Second passage through *Glossina morsitans.*

Mean.	σ	N.
19·4270	2·0186	462
23·3668	1·9566	38

$p_2 = -1{\cdot}1020.$ $P = {\cdot}75$

CHART XXVI.



SLEEPING SICKNESS COMMISSION. Royal Society's Report.

T. brucei XXV., after 3rd passage through *Glossina morsitans*.

Mean.	σ	N.
21·199	2·2425	500

$P = \cdot 06$.

$\beta_1 = 0 \cdot 2289$. $\beta_2 = 3 \cdot 0981$.

ON THE NATURE OF THE DOUBLY-REFRACTING PARTICLES SEEN IN MICROSCOPIC SECTIONS OF SILICOTIC LUNGS, AND AN IMPROVED METHOD FOR DISCLOSING SILICEOUS PARTICLES IN SUCH SECTIONS.

By W. WATKINS-PITCHFORD, M.D. (Lond.), F.R.C.S.,

and

JAMES MOIR, M.A., D.Sc., F.R.S.S.A.

Historical. In the Report of the Transvaal Medical Society to the first Miners' Phthisis Commission[1] it is stated that "by careful focussing it is quite easy to distinguish (in a section of silicotic lung) in the aggregations of pigment many minute angular transparent refractile particles. These are particularly numerous in the central parts of older nodules, and in the areas of advanced fibrosis." No mention is made of the use of the polarising microscope.

In 1910 Mr. Max Weber, the Curator of the Johannesburg Geological Museum, pointed out to one of us that the behaviour of some of the polarising particles in silicotic-lung-sections differed from that of small particles of quartz in that they polarised more strongly.

In 1911 Dr. J. L. Aymard, in a communication to a local technical journal[2], stated:—"Recently in investigating the dust microscopically, and also lung specimens kindly lent by Dr. Mitchell, a doubt has arisen as to the quantity and position of the quartz. Dr. J. Moir discovered that only certain portions of the supposed quartz showed with polarised light, and that the pigmented larger masses generally taken for quartz dust presented none of the characteristics of quartz, but he has considerable doubt as to whether the particles which shine in polarised light are really quartz."*

* This communication was accompanied by two photomicrographs, one of which is, we believe, the first published photomicrograph of a section, showing lung-dust, taken by polarised light. This photograph was taken by one of us (J.M.) in September, 1910.

Dr. E. L. Collis[3] considers that the method of examining sections of pneumonoconiotic lung by polarised light was first used in an enquiry into the effect of inhaling plaster of Paris dust, the report of which was published in Paris in 1912. He remarks, however, that Greenhow, in 1865, used polarised light to identify silica in the dust recovered from a metal-grinder's lungs.

Dr. J. McCrae in 1913[4] separated the siliceous particles from a portion of silicotic lung by a process of "acid digestion" (digesting the tissue over a water-bath with strong hydrochloric acid and potassium chlorate). It was presumed that the process was without action on the silica, and although it might leave some refractory silicates unchanged it nevertheless dissolved practically all the other inorganic and all organic matter. The residual white deposit was thoroughly washed with water, by centrifugalisation and decantation, so as to remove all soluble salts, and was carefully dried at a low temperature. A thin emulsion of the powder so obtained was examined microscopically, and its morphological characters ascertained. The use of an analyser and polarising eyepiece "proved that the material was silica." The great majority of the particles were found to be of indefinite shape, and approximately 70 per cent. of them possessed a diameter of less than 1 micron. Only a negligibly small number of particles was seen whose longest diameter exceeded 8·5 microns, and the longest observed was 10·5 microns. This discovery, in addition to its scientific interest, has had an important bearing on improved methods of preventing the disease.

Character of images of mineral particles in polarised lung-sections.

The anatomical situation, the outline, and the dimensions of mineral particles visible by polarised light in sections of silicotic lungs, *when mounted in Canada balsam*, has received the special attention of one of us[5]. These observations were begun in the early months of 1912, and in July of that year great assistance in the tedious work of micrometric measurement was received from Dr. A. I. Girdwood. Some of the conclusions arrived at were mentioned in a lecture[6] delivered at Capetown on the 9th of February, 1914, before the Capetown Branch of the British Medical Association.

Microscopic sections of lungs from seven silicotic subjects, stained, and mounted in balsam, were examined by polarised light under a moderately high magnification (Zeiss obj. DD). The sections varied in thickness from 3 to 9 microns. When a doubly-refracting particle was observed it was first ascertained—by inspection at different foci—that it was actually in the substance of the section and not lying on either of its surfaces. The next step was to find in what position of the analyser the outline of the bright object was most distinct; the particle was then, if possible, sketched and measured.

All the foreign bodies dealt with were visible as objects of varying degrees of brightness when the Nicols were crossed; when the Nicols were parallel the majority of them were invisible, but many—when their positions as doubly-refracting objects had once been carefully noted—

could also be detected as faint outlines when viewed by ordinary light. The form of a particle visible both with crossed and parallel Nicols was not always identical under both conditions: this was more particularly the case with recognisably plate-like objects which lay obliquely to the optic axis, for these when polarised often gave the misleading appearance of slender, elongated particles.

In order to gain some knowledge as to the anatomical distribution, shape, and size of the doubly-refracting objects in the tissues, 101 of them, encountered consecutively, were examined; that is to say, each clearly definable particle detected in a field was brought in turn to the centre, its surroundings noted, its form sketched, and its dimensions ascertained by a screw micrometer. This method counteracted the natural tendency to select those particles which were most conspicuous or which possessed the most regular outline.

From the record of actual observations appended (Fig. I.)—which is copied from the notes made at the time—it will be seen that 29 of the clearly defined particles lay free between cells or fibres (A), 46 lay free amongst necrotic *débris* (B), 18 were contained within the bodies of cells (C) which were mainly fibroblasts and some of which were in the early stages of disintegration, 7 lay free in pulmonary alveoli (D), and 1 occupied the lumen of a small bronchiole (E).

An inspection of the sketches (some of which have not been drawn accurately to scale) will show that the prevailing outline was narrow and elongated with one or both extremities pointed; many showed a slight curve in their long diameter and some were elliptical; a common form was that of a very slender prism having its ends cut off at right angles or obliquely.

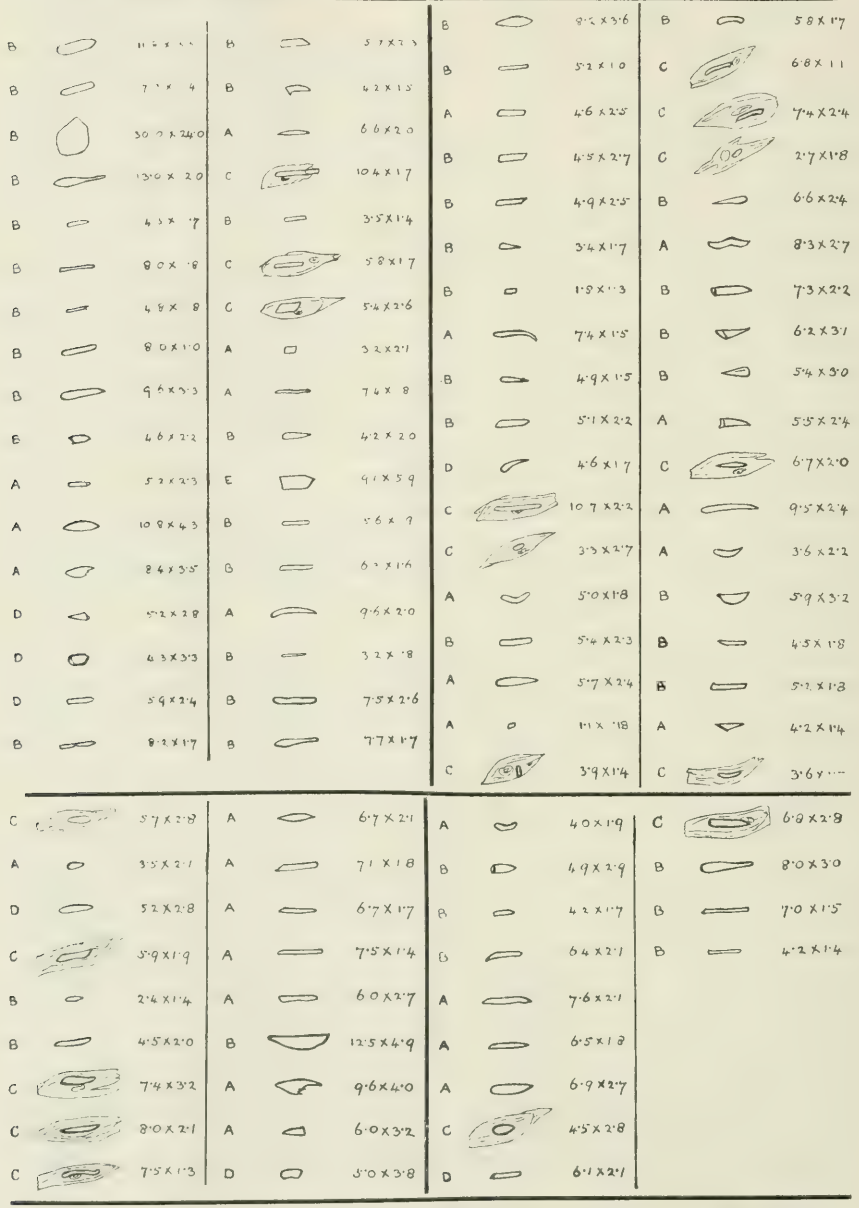
With regard to the dimensions of the particles it is to be remarked that the length of all of them (with the exception of the third examined) fell between 1·1 and 13 microns; in 14 it fell between 1 and 4 μ, in 39 it fell between 4 and 6 μ, in 29 it fell between 6 and 8 μ, in 12 it fell between 8 and 10 μ, and in 6 it fell between 10 and 13 μ. The mean dimensions of all the particles (exclusive of the abnormal third one) were 6·13 microns by 2·18 microns.

As necrotic areas in silicotic lungs frequently include the disintegrated remains of air-vesicles and bronchioles which have become devitalised by obliteration of blood-supply the presence in them of large particles does not necessarily indicate that such particles had actually penetrated the limiting wall of the alveoli and air channels. Inspection of specimens in the earlier stages of the condition shows indeed that all the doubly-refracting particles which quit the air spaces to enter the connective-tissue appear to be of small size and nearly all of elongated shape. It is probable, therefore, that the large, plate-like particle which comes third in the table had never actually entered the lung-tissue.

It is of special interest to note that some 18 per cent. of the doubly-refracting particles were found within the bodies of cells and that some

FIG. I.

Outline, superficial dimensions, and histological situation of 101 consecutive mineral particles visible in silicotic-lung-sections, mounted in Canada balsam and viewed by polarised light.



The capital letter against each figure indicates the situation of the particle, thus: A = free between cells or fibres; B = free in *debris* of necrossed tissue; C = within the body of a cell; D = free within an alveolus; E = free within lumen of bronchiole. The dimensions given are in microns.

of these "lodgers" were as long as 10 μ. This is one of the considerations which lead one to think that the size of the foreign bodies which gain access to the connective-tissue spaces of the lung is regulated mainly by a process of vital selection on the part of the phagocytic cells. The particles which are found lying free amongst the cells or tissue-fibres have probably all been conveyed thither within the bodies of phagocytes which have subsequently died and become disintegrated.

Another point suggested by these observations is that the presence of mineral fragments in the air vesicles and passages of deceased miners —who have not, in all probability, been exposed to mine dust for many months before death—is indicative of a tentative process of natural cure; such particles have probably been conveyed *from* the connective-tissue of the affected lung into the air spaces, and are therefore on the way to be expectorated.

It is to be borne in mind that the foregoing observations refer only to foreign particles possessing doubly-refracting properties. Furthermore, all the doubly-refracting objects detected in the sections are not included, but only those of reasonably definite outline; many were encountered which could not be sufficiently defined to permit of either their shape being ascertained or accurate measurements being made.

Some foreign bodies, obviously mineral in nature and which are optically inactive, can usually be detected by microscopic examination of the silicotic lung; such as these are not included in the foregoing description. It is also to be remarked that no consideration has been given to those mineral fragments which are present but which are invisible by both ordinary and polarised light when sections of silicotic lungs are mounted in Canada balsam.

Visibility of birefringent substances in microscopic preparations examined by polarised light

In view of the fact that grave doubt had been thrown on the siliceous nature of the birefringent particles visible in sections of silicotic lung, we have attempted a special investigation of their nature. The sections which had provided the material for the foregoing record, and many other similar sections, were critically examined. Before detailing the new observations which were made during this examination, it seems desirable to make a few remarks upon the conditions which affect the visibility of microscopic objects in polarised light.

(1) All transparent substances the visibility of particles of which is increased when the light which they transmit has passed through crossed Nicol prisms possess the property of *birefringence*, or, in other words, have two indices of refraction. These indices are known as the "ordinary" (ω) and the "extraordinary" (ε).* The amount of

* When a substance has only one index of refraction it is termed "isotropic", and the index is denoted by the letter μ (or sometimes n_D, when sodium light has been used). Again, biaxial (orthorhombic, monoclinic, and triclinic) crystals have three distinct indices of refraction which are denoted, in order of increasing magnitude, by α, β, and γ; in such cases $\gamma - \alpha$ is the measure of the birefringence.

divergence between these two indices ($\omega - \varepsilon$, or $\varepsilon - \omega$) is a measure of the strength of the double-refraction (birefringence) of the substance; the greater the difference between the two indices the more is the visibility of the particle increased in polarised light. Thus for rutile (one of the forms of crystallized dioxide of titanium and a strongly polarising substance) ω (in Na light)=2·616, and ε=2·903; the birefringence of this substance (2·903—2·616=·287) is therefore high, and very minute particles of this substance are therefore conspicuous in polarised light. For quartz, ω=1·544 and ε=1·553; this mineral has therefore a low power of double-refraction (1·553—1·544=0·009), and *small* particles of it should not therefore be markedly visible in polarised light.

(2) The degree of visibility of a birefringent particle, when viewed in polarised light, is also dependent upon the inclination of its crystallographic axis to the line of vision; if this be unfavourable—that is to say, if the crystallographic axis be parallel to either of the Nicols—the particles, though definitely birefringent, become quite invisible. The optimum inclination of the axis of the particle to the axis of either Nicol is that of 45°.

(3) Intensity of illumination is an important factor in the visibility of birefringent particles. Many which had previously been overlooked will become obvious immediately the illumination is increased.

(4) The polarising effect of birefringent particles in histological sections is more marked when artificial illumination is used and the room is darkened than when daylight is employed and the room is well lit.

(5) Birefringent particles which are possessed of an intrinsic colour (especially red or yellow) are more conspicuous, when daylight is being used, than those which are colourless.

Appearances of a silicotic-lung-section in polarised light.

When a section of silicotic lung, rich in birefringent particles, is examined with polarised light under comparatively low magnification (*e.g.*, Zeiss obj. AA with ocular 4) the appearance of the field is suggestive of the starlit sky. Under a higher power (*e.g.*, Zeiss obj. DD) the great majority of what previously appeared as tiny points and lines of light are resolved into bright, elongated images of, apparently, very definite outline—such as are depicted in the table of sketches (Fig. 1). A common form, which is not represented in the sketches, appears as a fine, irregular but usually sinuous, line, or two or three such lines converging at their ends and suggestive of a small cluster of down-barbules. Some of the points of light will be resolved into small, shapeless patches without definite boundaries, and others into minute groups of such patches—as though the translucence of a tiny window had been largely obscured by roughly smudging the glass.

An increase in the illumination, or in the degree of magnification, increases the apparent number of such elongated and shapeless objects.

Although they may be extremely numerous in the field it is very rarely that two or more elongated images lie at exactly the same focus: it is for this reason that photo-micrographs of such specimens, when taken at a higher magnification, entirely fail to reproduce the appearances seen by the microscopist.

The next point to be observed is that, like the stars of the midnight sky, the bright images are not all of the same colour; the majority of them are brightly white, some are distinctly pink or even red, a few are of yellowish tint, and, very occasionally, an image of pale greenish tint is observed. A very rough estimate—formed by enumerating the birefringent particles in several fields of different sections—showed approximately four coloured (three pink and one yellowish) to 128 white particles.

As already remarked, it is very rare to find, even in a very thin section, that more than one or two well-defined doubly-refracted images lie at the same focus. It was whilst verifying this observation that the following very important fact was noticed: As the objective is raised or lowered by the fine adjustment (a high-power objective being used) many of the bright images undergo an unusual amount of displacement, often accompanied by distortion of outline. When the attention is directed to the histological components of the section a lowering of the objective causes the fine anatomical elements to lose their definition without distortion of outline; and if they appear to move at all it is but very slightly from the outer part of the field towards the centre: this very slight centripetal movement of histological details, when it occurs, is uniform around the circumference of the field. Similarly, when the objective is raised from the position of accurate focus a very slight, uniform movement of the histological details may appear to take place, in the periphery of the field, in a direction away from the centre. If now the attention be turned to the birefringent particles in the section, whilst similar up and down movements of the objective are made, many of the bright images will be seen to move in *various* directions; some of them alter their outlines or appear to "flare out" on one side or the other into an ill-defined luminous patch, whilst some may appear to undergo a greater or less degree of rotation in the horizontal plane. This phenomenon must be held to indicate that the bright images of many of the birefringent particles—however clearly defined they may be—do not represent true projections of the outlines of the objects: they are, in other words, optical illusions. This conclusion has been verified by investigations to be subsequently detailed: it may be remarked, for the present, that the inference is supported by the fact that when the outline of a birefringent particle of *triangular* shape can be detected, *whilst the Nicols are parallel*, it is often found to be represented, when the Nicols are crossed, by a bright *acicular* image which is very much narrower than the particle itself: upward or downward adjustment of the objective will cause this acicular image to travel from one side to the other of the triangular object.

A considerable proportion of the elongated, doubly-refractile objects visible in silicotic sections do not exhibit this pseudo-parallax; their polarised images are approximately their true projected outlines, and an alteration of the focus will merely cause the image either to disappear or to become double. Many of such are of a singularly pure white, whilst some are coloured; their nature will be discussed hereafter (pp. 12-14).

Optical properties of silicotic-lung-dust which has been extracted by acid-digestion.

An examination was now made of a small quantity of the fine siliceous dust which Dr. J. McCrae[4] had extracted by acid-digestion of a portion of a miner's lung, and the following observations were noted.

When the fine powder was examined on a microscope slide, in the dry state, it was found that particles of a definitely prismatic or acicular contour were conspicuously infrequent, the great majority of the larger particles were angular scales of triangular (frequently cuneiform), or, most commonly, irregular outline. The contrast between the appearance of this specimen of separated lung-dust and that of the particles visible in polarised sections of affected lung is very striking, and is exemplified by comparing the sketch at the end of Dr. McCrae's contribution with the sketches given above (Fig. 1).

"Pseudo-birefringence."

When this unmounted specimen of lung-dust was viewed under crossed Nicols an apparently birefringent effect was observed, especially where the particles were closely aggregated; the phenomenon was not due to reflection from the upper surface of the particles, as it was present when the illumination was entirely by transmitted light. An investigation of this phenomenon led to the following observations:—

An unstained section of animal tissue, from which the paraffin-wax had been removed by xylene, and the xylene by absolute alcohol, was allowed to dry and then examined without a cover-glass. The whole section was found to behave optically as though it were birefringent—the tissues appeared light as contrasted with the dark background when the object was viewed through crossed Nicols.

The same phenomenon (which we call that of *pseudo-birefringence*) was observed when a piece of ground-glass was examined as an unmounted object; a convenient method of demonstrating it was to buff a small portion of the central region of a microscope slide by rubbing it with fine emery cloth. The effect of apparent polarisation is seen when either the roughened or the smooth side of the glass is uppermost, provided the ground surface is at the focus. If the ground surface is wetted with water the phenomenon, although greatly reduced, is still evident, and even when xylene is used it is not entirely abolished.

A thin layer of extremely finely-ground common salt—an isotropic, *i.e.*, non-birefringent, substance—when mounted dry, exhibits the phenomenon of pseudo-birefringence.

We conclude from these observations that this phenomenon of pseudo-birefringence is due to the scattering of the light by reflection from the numerous and irregular surfaces which it encounters during its passage through the object viewed.

An excellent example of the fallacy which the phenomenon of pseudo birefringence may give rise to is contained in a pamphlet, entitled "Dust and Miners' Phthisis," which has recently been issued by the Transvaal Chamber of Mines. The first photomicrograph in this pamphlet represents "fine mine-dust under polarised light," and is evidently an unintentional illustration of the phenomenon of pseudo-birefringence.

Silicotic-lung-dust in Canada balsam.

When a specimen of this silicotic-lung-dust was mounted in Canada balsam, it became, to a very large extent, invisible (Fig. 2). This phenomenon is due to the fact that colourless translucent particles embedded in an approximately colourless translucent medium are only visible if the refractive indices of the particles and the medium are different; the distinctness with which they can be seen is directly proportional to this difference. The refractive index for the ordinary ray (ω) in quartz is, according to Dana,[10] 1·544, and the refractive index of the Canada balsam employed was found to be 1·515.

In air (μ with ordinary daylight = 1·00029) quartz particles are, of course, extremely visible (Figs. 13 and 14), and they are also easily detected in water (μ with ordinary daylight = 1·33); the refractive indices of both these media are decidedly lower than that of quartz. In α-iodonaphthalene, the refractive index of which was ascertained to be, with daylight, about 1·77 (*i.e.*, decidedly above that for quartz), quartz particles are slightly more visible than they are in water.

The particles, and aggregations of particles, in Canada balsam, were, of course, rendered less inconspicuous when the substage condenser was lowered. When such a preparation was viewed through crossed Nicols the collections of particles appeared as clusters of faintly luminous points, whilst isolated particles gave no appreciable polarisation effect. With stronger illumination—such as direct sunlight reflected from the surface of a white glazed tile—the birefringent effect of all the particles was markedly increased.* It was noted, however, that even with the most favourable polarisation conditions only very few images could be detected which corresponded to the slender and elongated objects which were so abundantly visible in balsam-mounted sections of the same lung as that from a portion of which the dust had been extracted. This important observation suggested three conclusions:—

* With reference to this method of enhancing the polarisation, it is to be remarked that its efficiency is partly due to a slight polarisation of the light—due to reflection—before it reaches the microscope. When this source of light is used some of the particles appear darker and others lighter than the field; in the former case the two polarisations—(1) of the field, and (2) of the particles—are of opposite kinds, and in the latter case they are additive.

(1) That many of the particles of *quartz* incarcerated in a silicotic lung are more favourably circumstanced for visibility by polarised light than when they are free from the tissues.

(2) That many of the particles visible in the polarised lung-sections were of minerals more highly birefringent than quartz, and that they had been destroyed by the treatment to which the piece of lung had been submitted.

(3) That there is a considerable amount of quartz in the miner's lung which is not detectable in balsam-mounted sections when examined either in ordinary or in polarised light.

All three of these conclusions became justified by subsequent observations.

The optical properties of mine dust.

Samples of dust separated from Witwatersrand mine-air, and not from silicotic lungs, were now examined. The fine dust which floats in the air of the workings after the reef has been blasted was entangled by aspirating the air through a tube packed with pure, crystallised sugar; the sugar was dissolved out (using 5 c.c. of water per gram of sugar) and the solution allowed to sedimentate for a period calculated on the basis of twenty minutes for a depth of 5 cm.[7] The supernatant liquid, which was now free from the coarser particles, was centrifugalised, and the deposit thus obtained was submitted to examination. It was found to resemble, in its general characters, silicotic-lung-dust (separated by acid digestion), but included also many minute carbonaceous fragments. It consisted mainly of roundish or triangular particles of quartz. These particles varied in diameter from about 15 μ down to the limit of visibility (about ·25 μ with ordinary methods of illumination). Just as in silicotic-lung-dust, particles of elongated and acicular shape were conspicuously infrequent, and when such were detected they were often found to have a pink or yellowish colour and always to have a definite outline—this being frequently that of a square prism. When examined in the dry state and without any mountant the dust exhibited the phenomenon of pseudo-birefringence already referred to. When a specimen was mounted in Canada balsam the particles of quartz became almost invisible (Fig. 2), and when the light was polarised groups of *faintly* luminous points appeared,* and very occasionally a bright linear image—single, or like a small cluster of down-barbules—which altered its position, orientation, and shape with changes of focus. The few particles possessed of definite prismatic outline and inherent colour were found to be strongly polarising and to show *no* material alteration in their disposition or contour by varying the focus.

When such specimens of fine dust are allowed to deposit from an aqueous suspension on a microscope slide, and are then dried and mounted in balsam, it is obvious that the great majority of the plate-

* We find that English flint, when crushed to powder and examined in balsam by polarised light, presents a much more striking appearance than siliceous mine-dust (Figs. 3 and 4). Even minute fragments present themselves as close aggregates of brightly polarising points embedded in a very faintly luminous matrix.

like particles will have assumed a horizontal position. Such a position is the least favourable for polarisation, and it is this fact which, in part, accounts for the striking dissimilarity in appearance of polarised sections of silicotic lung in balsam and specimens of lung-dust or mine-dust similarly mounted. When a quartz scale lies obliquely to the line of vision (as is usually the case when it is incarcerated in lung tissue) it will appear, when polarised, as an elongated image usually of a spindle-shaped, acicular, or curved outline, and this image will alter its curvature and position with every change of focus.

Experimental production of acicular polarisation images in quartz scales.

That quartz scales when fixed at various inclinations in the tissues do actually give rise to these optical appearances was verified by the following experiment:—

A sample of purely siliceous dust was obtained from mine-air dust by boiling it for two hours with HCl and $KClO_3$, filtering, washing, drying and fusing at a red heat with $KHSO_4$ for half an hour—a little KNO_3 being added at first to secure destruction of carbon particles. The melt was now dissolved in dilute hydrochloric acid and filtered. The filtrate gave marked reactions for aluminium and titanium. The filtered dust was washed out and again suspended in water. The dust was now found to be apparently entirely free from all mineral particles except those of quartz, the plate-like outlines of which had not apparently been in the slightest degree affected by the treatment; they presented themselves as minute, roundish, angular scales. A suspension of this purely siliceous dust in water had a milky appearance. Eighteen c.c. of this suspension was injected into the marginal vein of a rabbit, and the animal died in about four minutes. Sections of the lungs, liver, kidneys, and spleen, when mounted in Canada balsam and viewed by polarised light, were found to contain markedly birefringent particles in the blood-vessels. All of them were small, some appearing as faintly luminous points and patches, but many having bright images of definite elongated, spindle-shaped, or acicular outline.

A simpler but equally effective method of demonstrating the same phenomenon was by adding a small quantity of the suspension of purely siliceous dust to freshly-shed blood in a capsule, allowing the blood to clot, and sectioning the clot. When a section of this clot was treated, by a process to be referred to subsequently (which dissolved the coagulated proteins), the liberated particles lost their birefringent characters, a change which appeared to be due to their now being able to settle down in the liquid medium and assume a horizontal position on the slide.

It is evident that it is only the qaurtz scales of comparatively large size which, when fixed vertically or obliquely, can yield images on polarisation; a certain minimum length of path within the particle is necessary to ensure polarisation of the rays. It should be remembered that the mine-dust, from which the material used in the foregoing experiments had been prepared, had been freed from all particles larger than 15μ in diameter; no particle of sufficient thickness to polarise brightly whilst lying in a horizontal position was therefore present.

Birefringent minerals other than quartz in silicotic lungs.

A study of published analyses of the Rand ore ("banket"), and of rock-sections of the latter, show that the dust resulting from the pulverisation of the auriferous rock must contain other birefringent minerals besides quartz; the absence of fragments of these accessory minerals from Rand silicotic lungs would, indeed, be surprising, and, as a matter of fact, their presence can be readily demonstrated in lung sections. That particles of these accessory minerals were not detected in Dr. McCrae's specimen of separated lung-dust was doubtless due to the character of the process by which such lung-dust had been extracted. The process consisted in digesting the tissue over a water-bath with hydrochloric acid and potassium chlorate for, we believe, three days, and there are very few minerals containing basic oxides in their composition which could resist such solvent treatment.

A comprehensive description of the minerals included in the banket was given by Prof. R. B. Young in 1909.[8] His researches, and those of others, have identified the birefringent accessory minerals of the reef as sericite, rutile, zircon, tourmaline, apatite, and chlorite. These may be separated from the quartz in the crushed rock by means of elutriation in heavy solutions, and when their particles are mounted as microscopic objects the identity of many of them with the particles already referred to as occurring in the silicotic-lung-sections can be verified, with this exception, that we have hitherto been unable to recognise apatite in lung-sections.

The occurrence of birefringent particles (other than quartz) in mine-air has been already referred to, and the presence of particles of similar shape, dimensions, and optical properties in silicotic-lung-sections makes it certain that, in the latter situation, they are not merely organic crystals.

Properties of the birefringent "accessory" minerals occurring in "banket"

Sericite is a silky, talc-like, potash-mica ($H_2KAl_3(SiO_4)_3$ or $KH_2(AlSiO_4)_3$), usually white, grey, or yellow in colour[9]. In all probability it belongs to the monoclinic system, and possesses a refractive index of about 1·59;[10] it is therefore but hardly visible in Canada balsam. Its birefringence ($\gamma - \alpha$) varies from ·039 to ·042, *i.e.*, about four and a half times that of quartz; the maximum white polarisation is therefore given by particles of little more than 8μ in thickness. In the case of quartz a thickness of nearly 40μ is necessary to achieve maximum white polarisation.

In a special rock-section of banket, kindly lent to us by Prof. R. B. Young, the sericite appears, in the matrix of the rock, mostly in large areas of striated structure; several areas are found, however, in which the sericite occurs as minute needles mingled with quartz.

When a small fragment of banket matrix was crushed in an agate mortar the powder was found to contain, in addition to quartz and the opaque minerals such as pyrite and chromite, large quantities of fine scales of sericite, together with crystals of rutile, zircon, chlorite, and (?) tourmaline. The sericite fragments in this particular specimen of matrix were actually more abundant than the quartz particles (Fig. 5).

It is almost certain that a large proportion of the bright white images which are seen by polarised light in a silicotic-lung-section *of Rand origin* are produced by sericite. A characteristic image of a sericite particle in such circumstances is a fine, bright white line, often curved or slightly sinuous; the line will sometimes bend to one side or the other and alter its apparent curvature with changes of focus.

Rutile.—A native form of titanium dioxide (TiO_2) which occurs in granitic rocks, more especially in the quartz veins. It takes the form of tetragonal, prismatic crystals (Fig. 6) with a good prismatic cleavage. The colour usually varies from reddish-brown to black,[9] but the variety which occurs in banket is of a rather pale yellow.

It is highly refractile, the mean of the refractive indices in three directions at right angles to one another being 2·712 (Dana, p. 168),[10] and it is therefore plainly visible in Canada balsam. It is also extremely birefringent ($\omega = 2{\cdot}616$, $\varepsilon = 2{\cdot}903$, $\varepsilon - \omega = {\cdot}287$, *i.e.*, about 32 times that of quartz). It polarises to the maximum white colour when only $1{\cdot}2\mu$ thick. The thickness and breadth of the prisms are the same. The crystals yield polarisation-colours when more than 2μ thick—*e.g.*, reddish at 2 and 4μ, greenish at about 3μ. An unusually thick particle in a lung-section appeared under polarised light as a fine straight line of electric blue colour. It may be remarked that quartz particles do not yield polarisation *colours* unless they are at least 50μ thick.*

In silicotic-lung-sections the mineral appears most commonly as an obliquely fractured prism, four-sided and often of considerable length. If the prism has not been fractured it appears to be terminated by bluntly-rounded—but really pyramidal—ends. Several of the sketches included in the series of 101 polarised images of silicotic lung particles (Fig. 1) are evidently of rutile crystals. Short fragments of rutile prisms are not infrequently detected in lung-sections, appearing under polarised light as small, clearly-defined particles of ruby tint. Owing to their high refractive index particles of rutile are easily detected in

* When the crystal is placed in its position of maximum birefringence the formula $b \times (-) = \lambda$ holds—in which b = birefringence, $(-)$ = thickness in microns, and λ wave-length (in microns) of the colour extinguished by interference. As the thickness of the crystal increases, λ gradually increases also, and the maximum white polarisation occurs when $\lambda = 0{\cdot}35\mu$, *i.e.*, the wave-length of light at the limit of visibility in the violet. When λ increases still further, *colours* are produced. Thus when $\lambda = 0{\cdot}4\mu$ (blue-violet absorption) the complementary colour, yellow, is seen; when $\lambda = 0{\cdot}48\mu$ (blue-green absorption) the complementary colour, orange, is seen; at $\lambda = 0{\cdot}52\mu$ (mid-green), crimson is seen; at $\lambda = 0{\cdot}6\mu$ (orange), indigo is seen; and at $\lambda = 0{\cdot}7\mu$ (red), green is seen.

When λ becomes larger than $0{\cdot}75\mu$ the fundamental absorption goes outside the visible spectrum, and the colours, which are repeated in the above order, are then due to *submultiples* of λ, and are of a mixed and impure nature.

such sections without the aid of polarised light; the polariscope is, however, a great aid to rapidly locating them.

Dana[10] (p. 345) illustrates a very characteristic arrangement of rutile crystals, two, or sometimes three, being attached by their ends and being inclined to one another at an angle of about 120° (Fig. 7). Typical examples of this geniculate form of rutile crystals have been discovered by us in silicotic-lung-sections on three occasions, and have been frequently met with in the dust from mine-air (Figs. 8 and 9).

Zircon.—Native zirconium silicate ($ZrSiO_4$) occurs as a primary constituent of many igneous rocks, but, as a rule, only as minute crystals.[9] The mineral shows a wide range in optical characters, a fact which suggests the existence of modifications of zircon. It appears as short tetragonal prisms similar to those of rutile, the length, however, being only about twice the breadth (Figs. 10 and 11). The mean of the refractive indices is 1·952, and the mineral is therefore easily visible in Canada balsam ($\mu = 1{\cdot}52$). Its birefringence is about seven times that of quartz (ω - 1·931, ε 1·993, $\varepsilon - \omega = {\cdot}062$). The maximum white polarisation is not given unless the particle is at least 5μ thick, and smaller crystals appear of a pale greyish-violet colour in polarised light.

Zircon crystals, although not uncommon, are much rarer in silicotic-lung-sections than those of rutile. They usually appear of a *pink* colour in ordinary light. They are encountered from time to time in mine dust (Fig. 12).

Tourmaline is a complex boro-silicate of aluminium having wide differences in chemical composition[9]. The range of the refractive indices is given by E. A. Wülfing[9] as follows: ε (Na) = 1·612 to 1·651, ω - 1·631 to 1·685.

The habit of the mineral is almost invariably prismatic with deeply grooved prism faces. "The material ranges from colourless to jet-black in colour and may be any shade of red, yellow, green or blue." Common black tourmaline is abundant in granitic and metamorphic rocks.

Tourmaline particles occur fairly frequently in silicotic-lung-sections, but are difficult to detect. The prismatic crystals are faintly visible as pale bluish-green objects which possess a birefringence about twice that of quartz. The maximum white polarisation is not given unless the particle is about 18μ in thickness, and consequently the polarisation of such particles as occur in lung tissue is usually very feeble.

Chlorite.—($Mg\ (AlSiO_4)_2$) is a mineral of pale greenish tint which is also present in the banket. It is with difficulty differentiated from quartz owing to its birefringence being even less, and its green tint not being obvious in minute fragments. In silicotic-lung-sections it is probable that it will be but rarely distinguished from quartz, as its refractive index is but slightly higher; the possession of crystal faces may, however, be of assistance in distinguishing it from quartz fragments, for the latter possess them but very rarely, if ever.

Relative proportion of quartz particles to other birefringent particles in silicotic lung and mine-air respectively.

The question now arose whether the proportion of birefringent particles visible in a polarised lung-section, and *which are not quartz*, is greater than that of their occurrence in the fine dust of mine air. Samples of such dust which were examined in the dry (unmounted) state were found to comprise about four hundred translucent feebly-polarising particles to every one which definitely polarised. We had therefore to ask ourselves, supposing a particular field in a lung-section contained twenty-five particles which definitely polarised in a manner unlike quartz fragments, whether we were to infer that there were probably some ten thousand particles of quartz in the same field—the presence of the vast majority of which was concealed by their unfavourable inclination, their low birefringence, and the fact that they were embedded in balsam and obscured by the tissue elements?

It seemed not unreasonable to suppose that an undue proportion of *elongated* particles (such as those of tourmaline, sericite, rutile, and zircon) would be found to have been deposited in the lung tissues owing to the presumed preference of phagocytic cells for engulfing and transporting elongated bodies. To elucidate this question it was necessary to devise some means of destroying the tissues of a silicotic lung without at the same time affecting either the quartz or the accessory (birefringent) minerals; by such means the true relative proportions of these extraneous particles in the silicotic lung could be ascertained. We have already pointed out, when discussing the birefringent accessory minerals of the banket, that the process to which Dr. J. McCrae[4] had submitted silicotic-lung tissue (prolonged digestion with hydrochloric acid and potassium chlorate) had probably dissolved the more refractile particles while leaving the quartz untouched. The ordinary process of ashing the lung tissue was also found inapplicable, because the fusible salts proper to the normal tissues caused the whole residue to cake into a vitreous mass, from which the extraneous minerals could not be isolated in their original form. Both of the following methods, however, appeared to give us satisfactory results.

New methods of isolating extraneous mineral matter from pathological specimens.

A preliminary treatment to remove traces of fatty substances is necessary; the piece of silicotic lung is cut up into 2 mm. fragments, soaked in excess of acetone, left overnight, then soaked in ether for ten minutes, and finally dried at 100° C.

The first method consists in saturating the dried tissue in a hot concentrated solution of ammonium perchlorate containing some ammonium nitrate, drying in an oven, burning it, mixing powdered perchlorate and nitrate of ammonium with the carbonaceous residue and heating until the carbon has disappeared (the combustion takes place at a temperature far below red-heat, and consequently no formation of glassy masses occurs). The residue is now washed with hydrochloric acid previously diluted with 10 parts of water (the birefringent minerals are quite insoluble in this weak acid) to remove calcium phosphate and other constituents of lung-ash, centrifugalised, the deposit washed.

centrifugalised again, and gently broken up for microscopic examination. Ferric oxide (resulting from the combustion of pyrites) is insoluble in the weak acid, and therefore remains admixed with the mineral matter.

The second method is to heat the fragments (after preliminary extraction in acetone and ether, and drying) in dilute *nitric* acid. At first the acid should be of the strength of 1 in 3 and the temperature should be kept below the boiling point. Much nitrogen is at first evolved; this is due to the decomposition by heat of the diazo-compounds formed from the amino-groups of the proteins by nitrous acid.

After the evolution of nitrogen has ceased the strength of the acid is increased to about 2 in 5 and the mixture is now boiled until no fibres remain. The deposit in the liquid now consists of the extraneous mineral matter and the carbon (soot) from the lung tissues. This is filtered off, washed, scraped off the filter-paper, and gently ignited until it is of pure white colour.

Specimens of silicotic-lung-dust extracted by both these processes have the same general microscopical appearances as those of Dr. McCrae's specimen previously referred to; particles possessing a marked birefringence are, however, far more common; it is evident that the birefringent particles of the "accessory" minerals, which are so easily detected in silicotic-lung-sections, have not been dissolved by the treatment (Figs. 13 and 14).

The relative numerical proportion of the quartz particles to the particles of more refractile minerals in these specimens of lung-dust is very large, *being, to all appearance, about the same as the proportion in fine mine-air dust stated on page* 15.

We conclude, therefore, that *little, if any, physiological selection takes place, either in respect of the chemical nature or the shape of the mineral particles which are deposited in the lung tissues; the only physiological selection which appears to occur is in the matter of size.*

This important conclusion is, of course, at variance with a commonly held belief that pulmonary silicosis is due to the invasion of the tissues of the lung by acicular particles of quartz. It has become obvious that not only amorphous particles of quartz but also particles of other moderately hard and insoluble minerals are liable to be carried into the lung tissues—whether they are equally deleterious is, however, one of the problems of the future.

Size of quartz particles in silicotic lung.

The great majority of the quartz particles in such specimens of lung-dust are less than 2 μ in diameter; none of them have been found to exceed 14 μ, and particles of such a size as this are relatively very rare—say, 1 in 100,000. Very occasionally an acicular or elongated-wedge-shaped particle of quartz is found, but they are of very much rarer occurrence than the prismatic crystals of rutile and the elongated fragments of other accessory minerals.

A record of the dimensions and shapes of 120 particles of lung-dust, separated by the processes which have just been described, has already

been published.[11] The results will now be briefly referred to; they may be instructively compared with those given in the earlier part of this communication and which were obtained by a study of the particles as they appeared in polarised sections of silicotic lung.

The particles of separated lung-dust were scrutinised, successively and without selection, in a specimen mounted in air and viewed by ordinary light. Of the 120 particles, 117 were quartz, 2 rutile, and one was probably a tourmaline fragment; the true ratio of the quartz to the more birefringent minerals was, however, almost certainly much higher, probably 400 to 1.

The following is a summary of the "average diameters" of these 120 particles—the "average diameter" being the geometric mean of the length and breadth of each particle:—

15	particles	less than	$\frac{1}{2}$ micron
16	,,	about	$\frac{1}{2}$,,
12	,,	,,	$\frac{3}{4}$,,
30	,,	,,	1 ,,
15	,,	,,	$1\frac{1}{2}$,,
13	,,	,,	$1\frac{3}{4}$* ,,
11	,,	,,	$2\frac{1}{4}$,,
3	,,	,,	$2\frac{3}{4}$,,
3	,,	,,	$3\frac{1}{2}$,,
1	,,	,,	4 ,,
1	,,	,,	$5\frac{1}{2}$,,

There appeared to be some selection of particles about 1 μ in average diameter. The mean of the average diameters of the 120 particles was 1·2 μ. The great preponderance of relatively small particles in the specimen examined was indicated by the fact that for every particle of an average diameter of 8 μ or upwards there were 2,000 which were smaller.

The demonstration of occult quartz in lung-sections.

The observations which we have now detailed showed us that the microscopic appearance of polarised sections of silicotic lung affords by no means a true picture of the content of the section with respect to the extraneous mineral matter; it now remained for us to devise a method which would disclose for microscopic examination the presence and situation of the large quantity of quartz which is ordinarily undetectable in silicotic-lung-sections.

The method which we have selected as being quite successful depends upon the destruction of the section by means of cold strong nitric acid. When the process is carried out with due attention to detail the hitherto invisible particles of quartz are plainly disclosed, and retain

* This was misprinted in the published report[11] as $2\frac{3}{5}$.

approximately the distribution which they had in the original section. The following is the technique which we recommend:—

(1) A section is cut from the paraffin block, fixed to a slide in the usual manner, stained, mounted in xylene, and examined under the microscope.

(2) A suitable field is selected and the exact position of the slide noted from the stage-micrometers. The sub-stage condenser is now removed and a grease-pencil mark is made on the under surface of the slide in order to indicate the situation of the selected field.

(3) The cover-glass is now slipped off and the xylene is removed by means of benzene followed by ether.

(4) By means of a sharp pocket-knife all the section is scraped off the slide excepting that part immediately overlying the grease-pencil mark. The area of the section thus left should be about 2 mm. square.

(5) A circle of molten paraffin wax (m.p. 58°C.), about 15 mm. in diameter, is now made on the slide with the remnant of the section at its centre.

(6) The slide is now placed in the blood-heat incubator on a small stand which is carefully levelled by means of a good spirit-level.

(7) Three drops of yellow, fuming nitric acid are deposited, without splashing, on the section-remnant by means of a capillary pipette fitted with a rubber teat. The slide on its stand is now loosely covered with an inverted dish so as to protect it from dust, but not so as to exclude air; the specimen is now allowed to remain until the acid has dried up. Sooner or later the acid will have spread over the whole area enclosed by the wax ring. The slide must on no account be disturbed until the acid is quite dry.

(8) The wax ring is now removed and the slide is cleaned with a piece of soft cambric as close up as possible to the section-remnant; this removes the dissolved material which has been deposited over an area which is about forty-five times that of the area of the section-remnant.

(9) A wax ring is again made around the specimen, and this is returned to its levelling stand in the incubator. A further three drops of nitric acid are now applied *with the greatest care* to the surface of the slide within the ring; they should be deposited as far away as possible from the position of the section-remnant.

(10) The specimen remains absolutely undisturbed until the acid has again entirely dried.

Almost the whole of the organic matter is destroyed by this method; a trace of gelatinous material is left, and this serves to attach the mineral particles to the slide. The particles of carbon are not destroyed

The results obtained during the progress of one of these experiments have been photographed at each important stage of the process (Figs. 15, 16, 17, and 18). These photographs enable those who have not the opportunity of repeating the work to realise what an enormous number of quartz particles may be concealed in the field of a silicotic-lung-section: the contrast between the polarised field of the section before treatment (Fig. 15) and the same field, viewed by ordinary light, after treatment (Fig. 17), requires no emphasis.

The photographs also demonstrate that the deposits of carbonaceous matter (pigmented areas) are an accurate guide to the position of the occult quartz particles.

Rapid method of rendering mineral matter visible in microscopic sections.

The foregoing method need only be employed when it is of importance that not the slightest disturbance of the mineral particles from their original situation shall take place. For rougher work in the demonstration of occult mineral matter in sections we recommend the use of strong hydrobromic acid (about 30% HBr gas, specific gravity 1·38).* The section must not be mounted in Canada balsam, but merely cleaned with, and mounted in, xylene. After the position of the field to be examined for occult silica has been recorded by means of the stage micrometers, the cover-glass is slipped off, the xylene removed by alcohol, and the specimen dried; in this condition the section is opaque and pseudo-birefringent. The cover-glass is replaced, the slide restored to its place on the stage, and a *small* drop of the HBr is deposited at one side of the cover-glass. As the acid spreads into the specimen it destroys the stain and renders the tissue transparent (hydrolysing the tissue to a material resembling gelatine), so that occult silica becomes visible. There is a danger, however, that artificial birefringent objects (crystals of hydrobromides of amino-acids) may be created at this stage. If therefore it is desired to exclude this possible fallacy the slide should be removed from the microscope-stage and cautiously warmed over a micro-burner until the organic tissue disappears, without, however, causing the liquid to boil, which would cause great displacement of the contents. The mineral matter is then left in a homogenous fluid medium, and the specimen may be examined in ordinary light for quartz and in polarised light for fragments of accessory minerals.

It will be noticed that the section (Fig. 15), which is illustrated in the series of photographs, shows a condition of marked perivascular fibrosis, the extraneous mineral matter being incarcerated mainly in the new connective tissue which has developed around an artery and vein. It is in this relatively early stage of silicosis that extensive deposits of

* The refractive index of this HBr is low (=1·42), so that even minute mineral fragments are conspicuous. Hydrobromic acid is chosen because it has a high boiling point, and does not fume appreciably or cause evolution of gas under the cover-glass; nitric acid is unsuitable for the latter reason. Sulphuric acid causes charring and too dark a coloration. Hydrochloric acid is destructive to microscope objectives owing to its fumes. Perchloric acid may be satisfactorily substituted for hydrobromic acid.

occult silica can be most strikingly disclosed: in the later stages, when the pulmonary alveoli have become entirely obliterated by the overgrowing connective tissue and the substance of the lung has lost most of its anatomical characteristics, the deposits appear less concentrated and less definitely localised.

"Fibrotic consolidation" not associated with unusually large deposits of mineral matter.

The condition which has been described by one of us [12] as "fibrotic consolidation" of the gold-miner's lung is characterised by the excessive development of "nodules" of young connective tissue around the vessels and air tubes of the lobules; the lesion arises in any stage of the silicotic process. The "nodules" thus produced increase in number and size, and may thus come to almost entirely replace the lung tissue of the lobe or portion of the lobe in which they have arisen.* Reasons are given (*loc. cit.*) for concluding that this very commonly occurring lesion in the silicotic lung is due to infection by the tubercle bacillus; that it is not due to any locally excessive deposit of quartz particles has now been ascertained. When sections of this young luxuriant fibrous tissue are submitted to destruction by either of the processes we have detailed above excessive amounts of quartz are not disclosed amongst its spindle-shaped cells, the deposits are still confined to their original positions around the small vessels and bronchioles.

The occurrence of birefringent mineral particles in the lungs of those not specially liable to silicosis.

The facts that the lung of the newly-born infant contains no silica, and that the amount of this mineral which can be recovered from the lung tissues increases with age,[13] suggest the probability that birefringent particles of extraneous origin would be detected in the lungs of all adults.

As it is probable that at least the greater part of the silica normally present in the adult lung has been inhaled, it may be presumed that its presence would be more obvious in the lungs of those who have lived in countries were sand-storms are prevalent.

We have examined the lungs of a farmer from the Orange Free State (who died at the age of 56 from a pontine tumour) and also those of a "bijwoner" (or tramp) from the Pretoria district, who died at the age of 55 from a disease which did not implicate the lungs. In both instances the relatives and friends stated that the deceased had never worked in the mines.

Microscopic sections of the farmer's lungs showed occasional small areas of perivascular fibrosis indistinguishable from those associated with very early silicosis, and in these areas a few doubly-refractile particles could be detected. In the lungs of the "bijwoner" occasional birefringent mineral fragments could also be found embedded in perivascular deposits of carbonaceous particles. In both cases the birefringent particles were mainly composed of quartz and sericite. On the other hand a large quantity of occult quartz was revealed when these

* When these nodules are divided by cutting through a silicotic lung they are usually found to disclose a coarse filigree pattern, and in such circumstances they have been referred to as "fibrotic figures."

specimens were destroyed by acid treatment. The quartz particles thus disclosed (Fig. 19) were of the same general appearance as those found in silicotic lungs—usually rounded or triangular scales—but were on the whole decidedly larger. The average diameter was about 3 μ, which is about twice that which we have stated to be the average diameter of silicotic lung particles.

Birefringent objects of other than mineral nature are sometimes encountered in histological specimens, and the recognition of such objects may prevent mistakes.

Birefringent objects of other than mineral nature.

Tissues which are ultimately fibrillar or laminar in structure (when prepared for histological examination) are definitely birefringent, and this property is enhanced when they have been stained with certain dyes such as eosin and methylene blue. Fibrous tissue, striped muscle fibre, and cuticularised epithelium are usually conspicuously birefringent, and we have noted instances of the same phenomenon in the nuclei of some of the component cells of malignant new growths—both connective-tissue and epithelial in origin.

It is almost needless to remark that fibrous tissue is the only inherently birefringent tissue likely to be encountered in sections of diseased lungs. It is to be observed that in this, as in all other instances of birefringence due to fibrillar structure, the polarisation is greatest when the long axis of the fibrils lies at 45° to the axis of either of the crossed Nicols, and that it disappears when it is parallel.

Animal hairs and fibres of cotton (either of which may be accidently included in a microscopic specimen) are strongly birefringent objects—doubtless in consequence of their fibrillar structure.

Birefringent particles of organic nature are sometimes encountered in mounted lung-sections. When the tissues have been coloured by van Giesson-haematein stain, for instance, minute, brown needles of fuchsine picrate, from 5 to 10 μ long by 1 to 2 μ broad, and occurring singly or in clusters, may be found. They are *easily visible in ordinary light*, and give a bright yellow polarisation under crossed Nicols. In the latter circumstances they might possibly be mistaken for sericite fragments; they are, however, easily soluble in alcohol, and are, as already stated, plainly visible by ordinary light. From chemical analogy it is probable that any basic dye is capable of yielding a crystalline and birefringent product with picric acid. Cholesterol, uric acid, and certain crystalline derivates of haemoglobin (such as haemin) are doubly-refractile but are not likely to be encountered in lung sections.

Microscopic specimens which have been mounted in balsam for several years are sometimes found to exhibit—scattered throughout the balsam—numerous bright acicular and plate-like images under crossed Nicols; they are either invisible or barely visible in ordinary light, and appear to be due to the formation of crystalline substances in the balsam. They disappear when the slide is warmed.

Quantity of extraneous mineral matter in different stages of the disease and in "normal" lungs.

Some attempt has been made to correlate the progress of the disease with the quantity of mineral matter disclosed when microscopic sections of lungs at different stages of the pathological process are destroyed by nitric acid, or rendered transparent with hot hydrobromic acid.

One somewhat surprising result of these experiments has already been referred to (p. 20), viz., that sections of lung tissue in which "fibrotic consolidation" has occurred, and which consist essentially of new and somewhat loosely compacted fibrous tissue, do not yield excessive quantities of mineral matter. Even by direct examination of such sections under polarised light the comparative paucity of acicular images is obvious, and, although the sections when destroyed by acid show perhaps 100 times as many non-acicular quartz particles as there were acicular images, yet this large number is far smaller than the number of particles liberated by acid from sections of less markedly fibrosed lung tissue.

The general statement appears justified that the greatest proportion of extraneous mineral matter is to be found in sections of lung tissue in the earlier stages of the silicotic process, and in tissue in which the fibrosis is still of microscopic character.

In the same way, a comparison of the amounts of extraneous mineral matter yielded by an "early silicosis" section, and by the "Pretoria tramp" section, gave the approximate result that the former contained about ten times as many mineral particles as the latter. The number of mineral particles in this latter "normal lung" was nevertheless calculated to be about three-quarters of a million per milligram of dry section,* or about 100,000 million in both lungs (assuming 140 grams as their dry weight). This is for so-called "normal" lungs, whereas the number in a silicotic lung proper must certainly be counted in billions, and in some cases may be as many as 20 to 30 millions of millions. Although the order of magnitude of these results can be depended upon it is obvious that such calculations are open to grave sources of error, and too much stress should not therefore be placed upon the actual figures. The largeness of the number of particles in "normal" lungs appears, nevertheless, to justify the remark that the term *silicosis* is scarcely specific enough for the disease—the condition is really one of *hyper-silicosis*.

* Dry sections varying in area between 70 and 80 sq. mm. and having a nominal thickness of 5μ weigh from 0.08 mg., in "normal lung", to 0.5 mg. in cases in which the silicotic process is far advanced.

SUMMARY AND CONCLUSIONS.

1. A detailed description of the use of polarised light for detecting mineral particles in sections of silicotic lungs, and of the phenomena observed, is given.

2. Those polarising mineral particles which, in sections, nearly always have an acicular appearance (*i.e.*, at least three times as long as they are broad) are now shown to consist of two main classes, *viz.*: (1) Tiny quartz scales which are held by the tissues in positions making a large angle with the horizontal plane of the section which contains them, and which thus give rise to the optical illusion of their being acicular particles; and (2) Crystalline fragments and crystals of the accessory minerals which accompany quartz in the reef of the Rand, mainly sericite-mica and rutile.

3. Those quartz particles which do not polarise to any appreciable extent are either particles of less than 3μ in diameter or if larger they are flattened and lie nearly horizontally. Since, like all quartz particles, they have very nearly the same refractive index as Canada balsam, they cannot be demonstrated in lung sections, either by ordinary or polarised light, without destroying the organic matrix in which they lie. These occult particles of quartz constitute over 99% of the total number of particles revealed by the processes we have employed; they are not acicular, but are essentially roundish or three-sided thin scales.

4. The bright polarising effect of the accessory minerals, such as sericite, is due to their high birefringence. It is fortunate that the mine-dust of the Rand contains such an appreciable quantity of markedly birefringent matter, since this serves to indicate the position of the deposits of quartz fragments and has thus assisted in elucidating the pathology of the disease.

5. A full discussion of birefringence, pseudo-birefringence, and refractive indices as applied to this problem is given.

6. The method of destroying lung tissue by means of hydrochloric acid and potassium chlorate was found to have resulted in the dissolving away of most of the accessory minerals present in silicotic lungs. Two new methods, which have not this objection, are described, together with a rapid process which is applicable to microscope sections of tissue.

7. The true contents of Rand silicotic lungs with regard to extraneous mineral matter, as thus revealed, were found to be identical—physically, chemically, and in the numerical ratio of the particles of quartz to that of particles of accessory polarising minerals—with the finer portion of Rand mine-dust. No indication of physiological selection of particles was found except in regard to size.

8. The amount of extraneous mineral matter in "normal" lungs (of elderly, non-mining South Africans) was found to be considerable, and to include a large proportion of quartz as well as some more birefringent minerals. The possible relationship between this condition of "normal" silicosis and liability to pulmonary tuberculosis is not discussed.

9. The amount of extraneous mineral matter present in silicotic lungs is much greater than in the "normal" lung. The maximum amount was found in cases in which the disease was in its earlier stages and before extensive disorganisation of the lung by "fibrotic consolidation" had occurred. An approximate estimate of the number of mineral particles present in the lungs is given.

10. Attention is drawn to birefringent tissues and birefringent objects which are not of mineral nature and which may be encountered in histological sections.

JOHANNESBURG,
September 14, 1916.

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APPENDIX.

SOME PHYSICAL AND CHEMICAL CHARACTERISTICS OF CERTAIN MINERALS AND OTHER SUBSTANCES.

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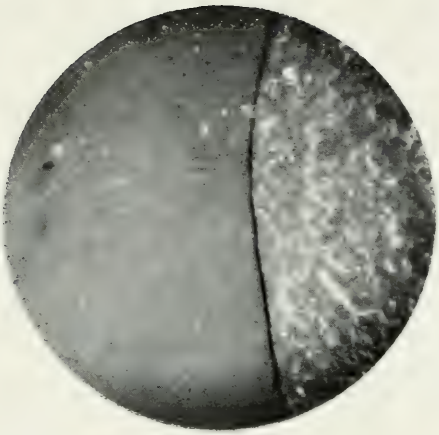


Fig. 2. Fine mine-air dust; polarised light: × 60. On the left of the field the dust is in Canada balsam, and on the right in air. The latter portion of the field shows the phenomenon of pseudo-birefringence.

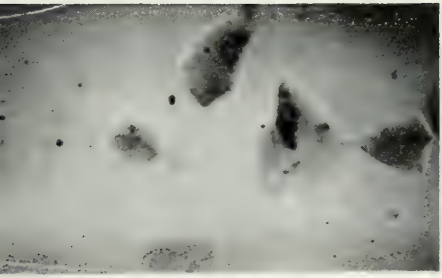


Fig. 3. Particles of English flint (as used by "knappers") in Canada balsam; ordinary light: × 50.

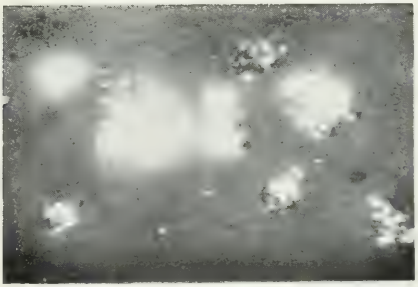


Fig. 4. Similar specimen in polarised light: × 200.

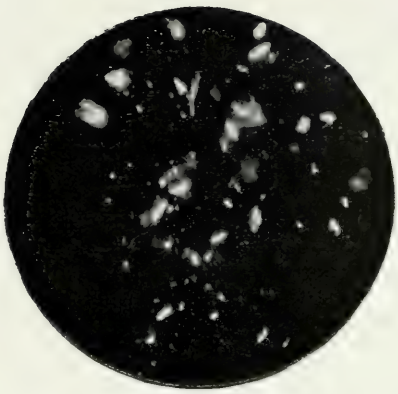


Fig. 5. Fragments of crushed matrix of banket ore in Canada balsam; polarised light; × 130. Sericite and rutile particles.

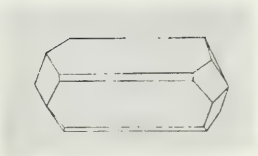


Fig. 6. Diagram of common rutile crystal.

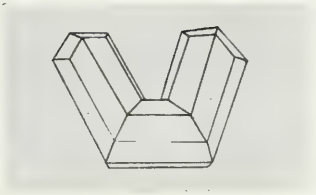


Fig. 7. Diagram of doubly-geniculated crystal of rutile.

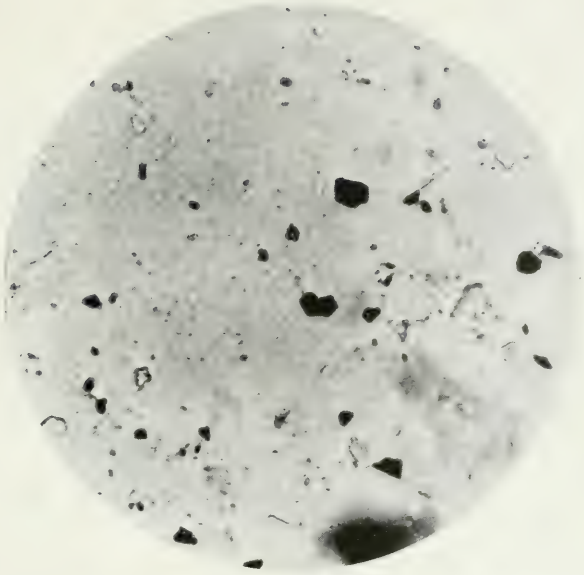


Fig. 8. Fine dust from mine-air: × 600. Near the centre of the field is a geniculated twin crystal of rutile.

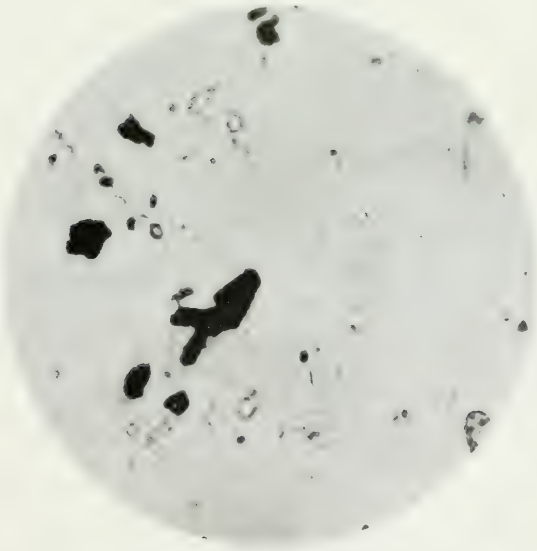


Fig. 9. Fine dust from mine-air × 700. Aggregation of five rutile crystals.

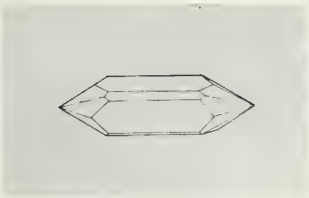


Fig. 10. Diagram of common Zircon crystal.

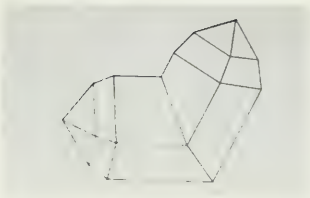


Fig. 11. Diagram of geniculated twin crystal of zircon.

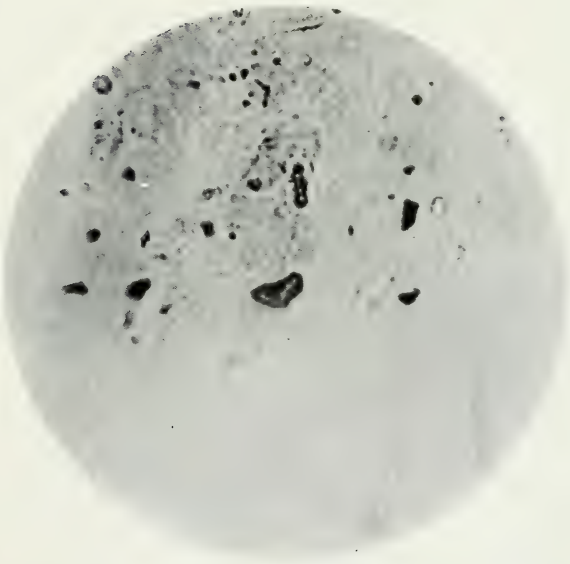


Fig. 12. Fine dust from mine-air: × 700. Near the centre of the field is a geniculated twin crystal of zircon.

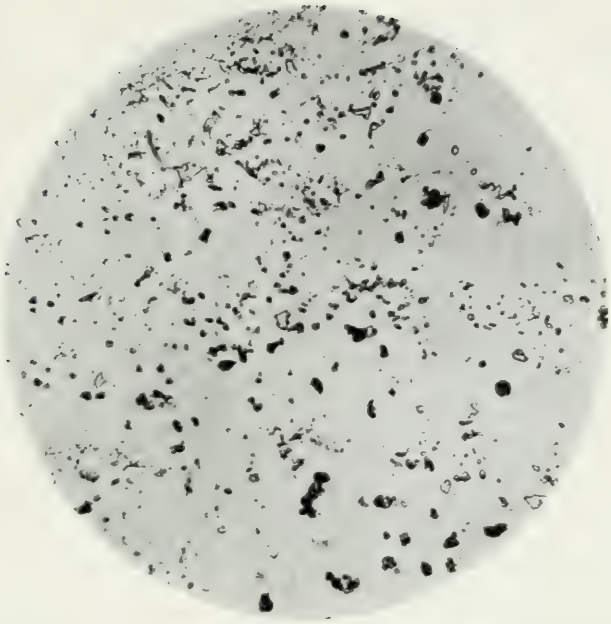


Fig. 13. Extraneous mineral dust extracted from silicotic lung; mounted in air; ordinary light; × 400.

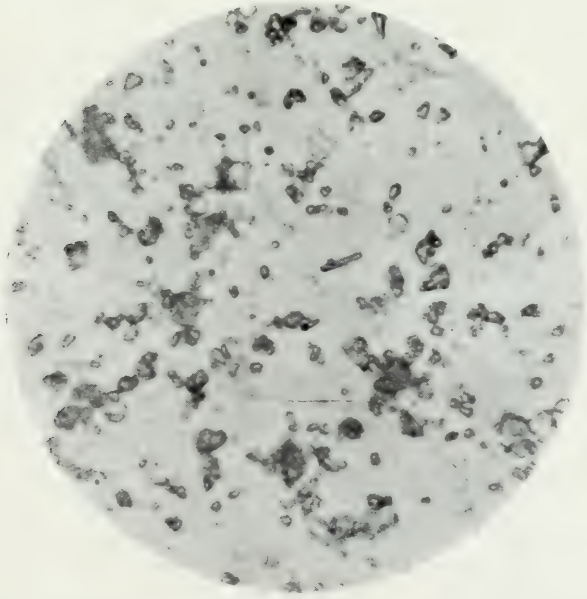


Fig. 14. Similar specimen; × 1000. The elongated object near the centre of the field is a rutile crystal.

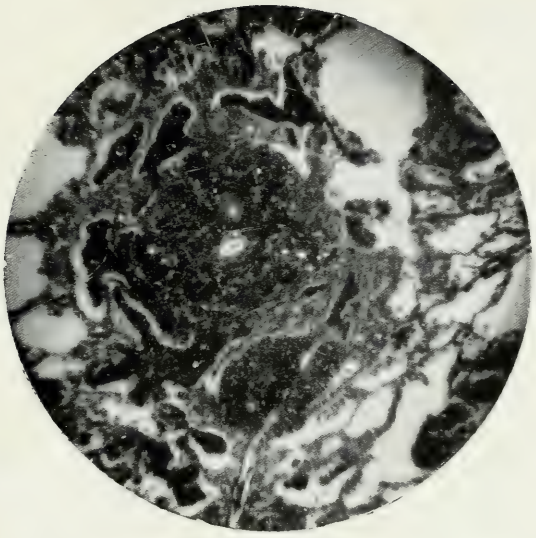


Fig. 15. Early pulmonary silicosis; polarised light: × 48.

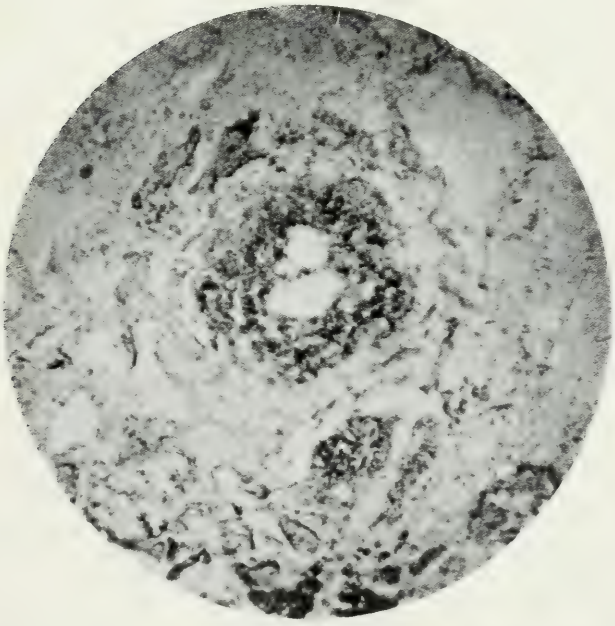


Fig. 16. The same field after first treatment with HNO_3; ordinary light: × 48.

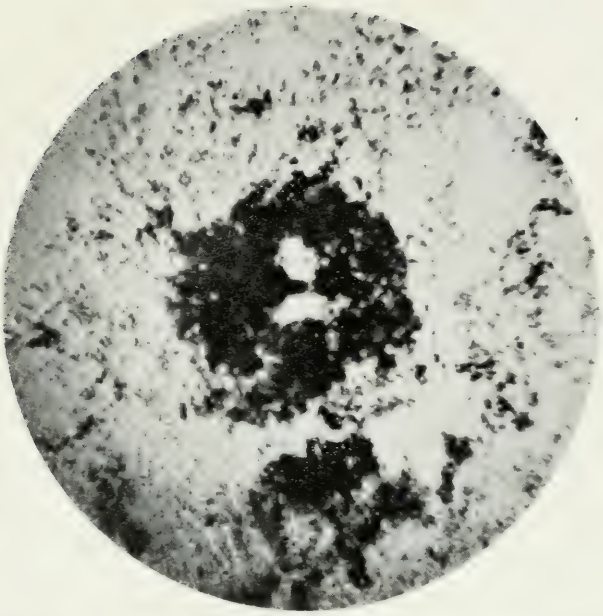


Fig. 17. The same field after second treatment with HNO_3; ordinary light; × 48.

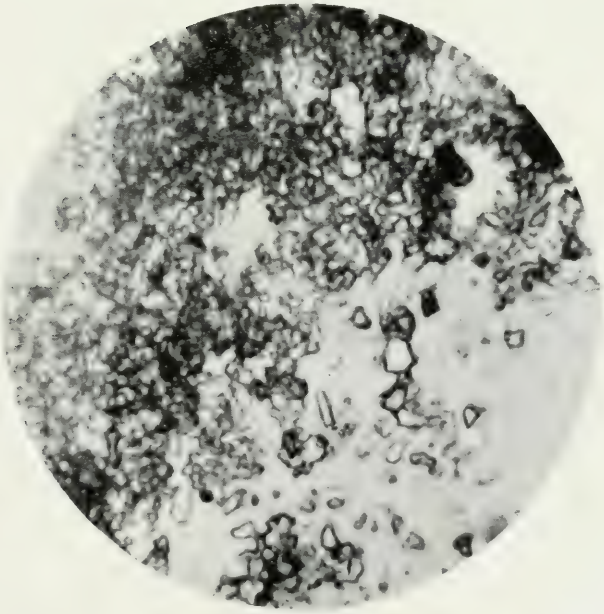


Fig. 18. Near the centre of the same field after second treatment with HNO_3; ordinary light; × 900.

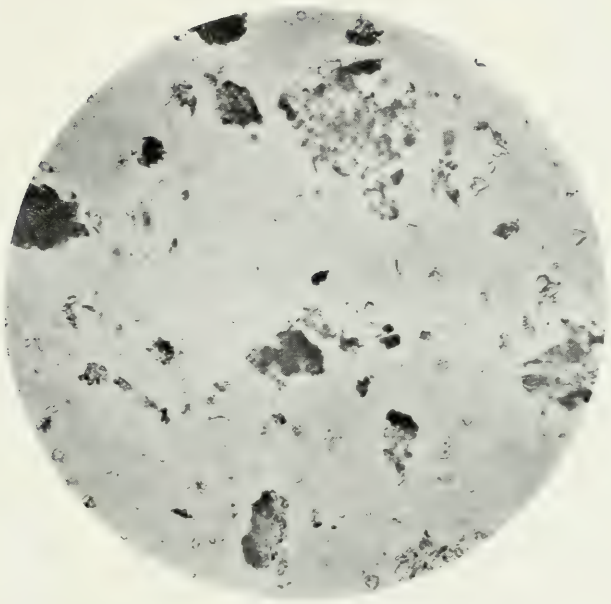


Fig. 19. Extraneous mineral dust extracted from "normal" lung tissue; mounted in air; ordinary light; × 240.

AN EXPERIMENTAL STUDY OF PROPHYLACTIC INOCULATION AGAINST PNEUMOCOCCAL INFECTION IN THE RABBIT AND IN MAN.

By F. S. LISTER, M.R.C.S., L.R.C.P.

This work was undertaken with the particular object of investigating the possibility of conferring upon man immunity against Lobar Pneumonia. It is almost certain, however, that such an immunity, if attained, would be found to extend to other diseases, *e.g.*, Pneumococcal Peritonitis and Pneumococcal Meningitis; it might, indeed, extend—though the grounds for such hypothesis are not so good—to some catarrhal conditions of the respiratory tract which result from pneumococcal infection. The Pneumococci concerned in this latter group do not belong to those classifiable strains so predominantly associated with the first three diseases above mentioned.

Lobar Pneumonia is a disease of great importance to South African mining communities; even in years of least prevalence it probably accounts for more than 30% of the total mortality amongst native mine labourers, and in the worst years it contributes 50%, or even 60%, of the total mortality.

Many of the native workers are employed for six months only, and the longest contract for such labourers is about one year; there is, however, a very marked incidence of Lobar Pneumonia amongst new arrivals. These two facts are of some importance, because an efficient immunity of only a relatively brief duration would accomplish its object as far as native mine labourers on the Rand are concerned.

I have succeeded in finding but two references dealing specifically with prophylactic inoculation against pneumococcal infections, either in man or animals; the records of attempts to devise or discover therapeutic agents constitute, on the other hand, a very considerable literature.

J. Brucker and P. Galasesco[1] recently reported the arrest of an epizoötic of Pneumococcal Septicaemia occurring amongst 500 guinea pigs. They inoculated the animals on three occasions with a killed broth culture of Pneumococci, and found that there were no further deaths after the third inoculation. In a similar outbreak during the previous year 160 animals had died out of 200 exposed to the infection.

The work of Sir Almroth Wright[2] and his co-workers (Drs. Parry Morgan, R. W. Dodgson, and L. Colebrooke) affords the sole contribution to the subject in so far as man is concerned.

The conclusions arrived at by Sir Almroth Wright and his co-workers are based upon investigations carried out at Johannesburg, Transvaal, at the end of 1911 and throughout 1912. Their work comprised chiefly—(1) The determination of the immunilogical changes in Natives' sera which were consequent upon their method of prophylactic inoculation (such changes being investigated by means of phagocytic mixtures and bactericidal tests *in vitro*); and, (2) The experimental inoculation of large groups of native labourers, and the evaluation of the immunity so conferred by a statistical consideration of the incidence of Lobar Pneumonia in inoculated and control groups.

It is unnecessary to review in detail the many conclusions and hypotheses set out in the record of their important piece of work—a work which entailed an immense amount of labour. In the light of certain biologic facts respecting the Pneumococcus, which have since been discovered, it is possible, however, to experimentally disprove such of their conclusions and hypotheses as refer to the production of demonstrable antibodies in the sera of pneumonic patients and in the sera of Natives inoculated with pneumococcal vaccine; and it is also possible to show that they very greatly underestimated the degree of immunity-response of which both African Natives and Europeans are capable.

They report, for instance, "that the agglutination reaction is with the pneumococcus only very irregularly obtained"; and, speaking of subcultures of the Pneumococcus from blood and lung-punctures, that "The vast majority of these gave no agglutination reaction with the blood of any patient. Moreover, in the case of those exceptional cultures which gave agglutination, we obtained the reaction not only with a large number of sera from pneumonic patients, but also with a number of normal sera. By consequence we put aside the agglutination test as one that could not be utilised for the purpose of estimating the effect produced by pneumococcus vaccines." So far is this from being correct that I hope to make it clear, subsequently, that at least 90% of pneumococcal cultures obtained by lung-puncture are agglutinated by the patient's own serum: that agglutinins are very constantly produced: that they can be demonstrated in the serum of both the rabbit and man as a result of inoculation with killed Pneumococci: that the phenomenon of agglutination is the surest and most accurate guide as to the progress of immunisation: and, finally, that agglutination never occurs with normal serum, provided the Pneumococci employed belong to that class capable of giving rise to Lobar Pneumonia.

In summing up the thousands of opsonic estimations which they undertook, and referring to the degree of immunity-response of which, as judged by this procedure, an inoculated Native is capable, they write—"We were able to satisfy ourselves that the doses of 200 to 300 millions which we were tentatively administering at the outset of our work elicit in the Native an opsonic response which brings him in the ordinary case up above the level of the uninoculated Native, and in the favourable cases up to the level of the Native who is making good immunising response against pneumonia. In other words, the opsonic response which is in favourable cases elicited in a Native by a dose of 200 to 300 millions of pneumococci would seem to be *not much below his maximal opsonic response**—assuming this to correspond to the best opsonic response met with in the Native pneumonic patient." An estimate of what is to be understood by "the best opsonic response met with in the native pneumonic patient" may be gathered from a foot-note, which is as follows:—"*That this level is sometimes higher than that of the uninoculated European* will be seen on referring to tables IV. and V. and noting that there were among eight pneumonic patients two who had a higher phagocytic count than any of ourselves."

On turning to the tables referred to in this foot-note we find that the example showing the greatest superiority of a Native patient's serum over that of the best European tested indicates an opsonic index of 1·9, and it is to be specially remarked that the cultures used in a great number of these experiments were chosen from those which gave some degree of phagocytosis both with normal European and normal Native sera in order that differential results might be obtained.

The authors, in discussing "The Racial Differences between the Tropical African Native and the European with respect to the Antibacterial Effect which their Bloods exert upon the Pneumococcus," make the following statements:—"That it is only exceptionally that the opsonic index of the Native who is making immunising response comes up to the *arithmetical mean of the pneumo-opsonic indices of the Europeans who served as controls in these measurements.* The full significance of this fact comes home to us only when we realise that it is, not so much the original content of a patient's blood in protective substances, as his capacity for generating these in response to microbic invasion which gives the measure of his power of combating infection. Once this principle has been grasped it becomes clear that there is furnished to us, in the comparison between the opsonic power of the normal white man and that of the Native who is making active immunising response, a better measure of the *Native's defect of resistance* than is afforded by the comparison between the opsonic power of the normal white man and that of the normal Native."

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* The italics in this and subsequent quotations are mine.—Author.

The authors deal at some length with the many difficulties which arise in opsonic investigation where the Pneumococcus is concerned, and say that they derived "a lamentably small harvest of suggestive and interesting facts."

It will be seen from the experiments which I detail later that the maximal opsonic response, not only of the Native pneumonic patient but also of the inoculated Native, is far greater than one would be led to assume from the foregoing conclusions of these authors. It is sufficient, for the present, to state that a culture of Pneumococcus which gives no phagocytosis with a normal European serum will, nevertheless, show a phagocytosis *in vitro* of from 500 to 3,000 cocci in 50 leucocytes (after only 15 minutes' incubation at 37°C.) with an homologous serum obtained from either a Native pneumonic patient (in the post-critical stage) or a suitably inoculated Native.

The prophylactic dose of from 200 to 300 million cocci (which was stated to be capable of producing a degree of immunity not far short of the "maximal opsonic response" for a Native) was subsequently increased to one of 1,000 millions; this increase was decided upon in consideration of the statistical results obtained from very large groups of inoculated Natives and their controls. There is no record, however, to show that the sera of Natives inoculated with this increased dose exhibited any greater rise of opsonic or bactericidal power than those previously treated with a dose of from 200 to 300 millions.

The work I now record will demonstrate that larger doses than 1,000 millions, even of a single strain of Pneumococcus, are necessary in order to give rise to demonstrable antibodies in a Native's or European's serum; and furthermore it will be shown that it is essential for practical purposes to immunise against more than one group of the Pneumococcus.

The authors further point out that "our opsonic measurements failed to tell us (*a*) what is the optimum dose of pneumococcal vaccine for general use in prophylaxis . . . (*c*) whether one inoculation ought to be followed up by another, and if so, how soon it ought to be followed up; and (*d*) whether a single hypodermic inoculation, or (*e*) the same aggregate dose of vaccine distributed in multiple hypodermic inoculations or (*f*) intravenous inoculations give the best immunising response."

In the following pages I deal specifically with these questions, and the recorded experiments will afford definite answers to each of them.

It would ill become me to criticise the conclusions of such a great authority as Sir Almroth Wright were I not very sure of my ground, and were I not also convinced of the necessity of attempting to overtake and rectify the erroneous impressions which must doubtless have been formed in the minds of many after a perusal of his Report. I am particularly indebted to Sir Almroth Wright for personal instruction

in his special and ingenious methods of technique: I have used them throughout this work, and for them and their originator I have the greatest admiration.

We have now to ask ourselves what are the fundamental discoveries in connection with this important subject which have been made since the completion of the Report referred to, and which have made it possible to demonstrate how widely divergent are the actual facts from the conclusions of Sir Almroth Wright and his co-workers? They are briefly as follows:—

(1) Pneumococci have been separated into distinct biologic groups: and,

(2) The immunity established in animals (including man) by the inoculation of a vaccine prepared from a member of any of these groups is specific only against such group.

Classification of Pneumococci into Distinct Biologic Groups.

During 1913, A. R. Dochez and L. J. Gillespie,[3] in America, and the author independently in South Africa,[4] classified Pneumococci into distinct biologic groups. Immunilogical and serological reactions were the basis of these classifications, and the phenomena associated with them were shown to be specific for each group.

A. R. Dochez and L. J. Gillespie, working in New York, differentiated Pneumococci into four groups, as follows:—

Type I. representing about 45% of all Lobar Pneumonia Cases.
Type II. ,, ,, 20% ,, ,, ,, ,, ,,
Type III. ,, ,, 14% ,, ,, ,, ,, ,,
(Pneumococcus mucosus)
Type IV. ,, ,, 22% ,, ,, ,, ,, ,,

Of group IV. they state, "Group 4 . . . consists of a heterogeneous collection of isolated individuals. . . . The study of cross protection and agglutination among members of the heterogeneous Group 4 indicates that this group comprises a number of distinct varieties of pneumococcus which cannot be related to one another by immunologic reactions. Culturally they are true pneumococci and manifest all the common characteristics of pneumococcus."

O. T. Avery[5] has investigated certain strains of Pneumococci which are agglutinated by Dochez and Gillespie's Type II. antiserum (corresponding to the author's Group "B"), and he finds that they are agglutinated "but incompletely and less promptly, often being delayed several hours." By means of absorption and protection tests he has shown that these atypical cultures possess group relationships among themselves and a common antigenic relation to the original and typical Type II. Pneumococcus. His work deals with 10 strains which he has placed in three sub-groups, viz., sub-group II.A, 4 strains; II.B, 3 strains; II.X, 3 strains. Antiserum of Type II. protects against sub-

groups II.A and II.B, but not against II.X which consists of a number of heterogeneous strains. From the practical point of view of prophylactic inoculation against Lobar Pneumonia this new and further subdivision of Group II. (my group "B") is not of great importance; members of these atypical sub-groups are apparently rare, and protection is afforded against 70% of them by the inoculation of a typical member of Group II. (my "B"). I note, further, that only four of these ten cultures appear to have been derived from cases of Lobar Pneumonia, and that none of these four belonged to sub-group II.X (the group against which Type II. antiserum affords no protection). It is true that this latter fact may be a mere coincidence, since the numbers are small; in view, however, of the additional information that one of the characteristics of this group is its "lower virulence" one may be at least allowed to speculate as to whether it is capable of giving rise to Lobar Pneumonia in man.

My classification is identical with the above in so far as groups I., II., and III. are concerned; these groups correspond respectively with the groups which I originally designated "C," "B," and "E," a fact which has received confirmation by A. R. Dochez at the Rockefeller Institute, to whom I have sent the desiccated spleens of mice infected with members of my various groups. Samples of immune serum, homologous with the Rockefeller Institute groups I., II., and III., were (through the courtesy of Messrs. Dochez and Gillespie) forwarded to the South African Institute, and investigation again showed that Nos. I. and II. corresponded respectively with my groups "C" and "B." With respect to serum No. III. I have not, as yet, been able to correlate it with my group "E," as no culture belonging to this group has been available for the purpose; the identification of the strain has, however, been achieved at the Rockefeller Institute, as already stated.

Dochez and Gillespie include in their Group IV. every culture of Pneumococcus which has not the immunilogical characteristics necessary to identify it with their groups I., II., or III.; they state, moreover, that they are unable to find any sub-groups amongst the individuals comprising this Group IV.

It is at this point that my classification departs very widely from that of the workers at the Rockefeller Institute. In the Transvaal, at any rate, there are a number of additional groups, and one of them (Group "A") is of great importance; members of this group are *more prevalent than those of any of the groups "C," "B," or "E,"* and cause a high case-mortality. The prevalence of this group has remained consistently high during 1913, 1914, and 1915.

My other groups, viz., "D," "F," "G," "X," and "Unclassifiable," are of less importance: they appear to vary in prevalence in different years, give rise to a relatively low case mortality, and, as shown in Table I., some of them have been without a representative in fifty consecutive pneumonic cultures. Further interesting features

which were disclosed whilst this Table was in preparation are—(1) The decline in prevalence of Group "B," and the increase in Group "C," during 1915, and (2) The discovery in 1915 of Groups "F," "G," and "X" which had not been hitherto met with.

My group "X" is composed of cultures of Pneumococcus which, although they are severally agglutinated by the patient's serum, are not agglutinated by the serum of any other case of Pneumonia. No cross reactions have been obtained amongst the members of this group, and in this respect it corresponds to Dochez and Gillespie's Group IV. As will be seen in Table I. only six such cultures were obtained out of a total of 96. The peculiar character of this group is, I think, not that each member is an unique specimen, but that on account of their rarity duplicates have not yet been met with.

The individuals which compose the group which I have designated "Unclassifiable" appear to give rise to Pneumonia of a clinically severe type, though of the four cases met with all have ended in recovery. The serum of the patient in these cases gives no agglutination with the homologous organism, neither is the organism agglutinated by any of the immune sera corresponding with my other groups.

Cultures belonging respectively to my "A" and "D" groups were investigated at the Rockefeller Institute, and Messrs. Dochez and Gillespie have informed me that they placed them in their Group IV. Now, Group "A" is the one most frequently found on the Rand, and in view of the fact that it has not hitherto been met with in America and Germany it must be provisionally considered to be peculiar to South Africa.

Although Group "A" is numerically important in South Africa the existence of other "local" groups, designated by me "D," "F," and "G," must be borne in mind. We are here faced with the anomaly that the classification devised by Dochez and Gillespie, although applicable to America, does not provide for the numerically most important of our local groups, as well as for several other distinct groups found in South Africa. What classification will ultimately be adopted it is idle to speculate, but it would appear necessary that it should embrace and distinguish all known groups of Pneumococci in whatever part of the world they may happen to occur.

A. R. Dochez and O. T. Avery[6] advance the following hypothesis to account for the presence in South Africa of strains unknown in America:—"The most likely explanation of this phenomenon seems to be that in South Africa among the whites, as in this country, there are a number of instances of Pneumonia due to organisms resembling the sputum Pneumococcus, the slightly virulent Group IV. of our classification. When such a Pneumococcus is communicated to the susceptible black its pathogenic history, in spite of its relatively low virulence, does not stop with the production of a single instance of Pneumonia, as it seems to among individuals whose racial immunity is

relatively high, but the organism is readily passed on to other susceptible blacks, and thus establishes itself in the less immune race as a permanently pathogenic type." They also state—"It is possible that in South Africa the new races arising among the natives may, after a number of generations, gain sufficient virulence to become highly infectious for the relatively immune white." It is of interest to refer, in connection with the latter suggestion, to an instance which I recently investigated[7] of the occurence of Lobar Pneumonia in wife and husband (Europeans), and in which the disease manifested itself in the husband within eleven days of the onset of his wife's illness. Both patients had been admitted to the Johannesburg General Hospital, and at the time of coming under my observation both had passed their crises; in each case, however, the serum agglutinated a culture of my Group "G" Pneumococcus, and did not agglutinate any other group. A culture isolated from the husband's sputum (by mouse-inoculation) proved also to belong to Group "G" and was fully agglutinated by my antiserum homologous with this group as well as by the serum of both the wife and husband: it was not agglutinated by any antisera homologous to my other groups. I was unable to secure a sample of sputum from the wife. Group "G" is uncommon amongst Natives, and still rarer amongst Europeans. Nine consecutive pneumonic cultures recently obtained from Europeans belonged without exception to Group "C." It therefore seems possible that this is an example of the rise of a new race of Pneumococcus capable of giving rise to Lobar Pneumonia in Whites; it is also highly suggestive of being an instance of case-to-case infection.

The strains of Pneumococci which I have isolated from cases of Lobar Pneumonia, by lung-puncture or blood-culture, during the years 1913-15 may be tabulated as follows:—

TABLE I.

Showing the different groups of pneumonia-producing Pneumococci, and the number of individuals which fell into each group, during three periods of investigation on the Rand (1913, 1914, and 1915).

Groups.	1913.	1914.	1915.	Totals.
A	9	12	16	37
B	5	5	1	11
C	1	3	15	19
D	3	3	0	6
E	0	2	0	2
F	0	0	7	7
G	0	0	4	4
X	0	0	6	6
Unclassifiable ...	2	1	1	4
No. of Specimens	20	26	50	96

Bearing in mind the fact that this Table deals with rather small numbers it is nevertheless to be observed that two groups account for over 60% of the cultures investigated in each year, and that Group "C" became second in numerical importance in 1915—a position which had been occupied by "B" in 1913 and 1914; only one specimen of the latter group was discovered in 50 consecutive pneumonic cultures investigated during 1915.

In order that the bearing of the experiments which are subsequently described may be rightly appreciated it is necessary, at the outset, to emphasise as strongly as possible certain fundamenatl facts, viz., (1) That the immunity established in animals (including man) when they are suitably inoculated with a vaccine prepared from a culture of any given group of Pneumococci is specific against members of that group and that group alone. (2) That serum obtained either from pneumonic patients (subsequent to the crisis) or from suitably inoculated animals (including man) will agglutinate organisms belonging to its homologous group, but that it fails to agglutinate, in the slightest degree, any members of other groups of Pneumococci.

The specific limitation of the agglutination phenomenon just referred to maintains also where phagocytosis is concerned; in the latter case, however, a word of caution is necessary. Cultures of Pneumococci used for investigating phagocytosis should, where immune sera are under consideration, be selected on account of their resistance to phagocytosis in the presence of normal serum. Although the differences which I shall record were quite apparent and very markedly in favour of the immune serum yet it is to be remembered that if a Pneumococcus that has undergone many subcultures on artificial media be used it will *usually* be found to give a high degree of phagocytosis in the presence of *normal* serum. It is true that the substitution of immune serum will bring about a still higher degree of phagocytosis—even to the filling of leucocytes to a point when it would appear impossible to crowd in a single additional Pneumococcus—yet the demonstration of the dominance of the immune serum over the cocci is less striking when cultures which are easily phagocyted in the presence of normal serum are employed.

It seems to me desirable to reiterate the absolute specificity of the immunilogical characteristics possessed by the various groups of Pneumococci, for the necessity of recognising this fact is not sufficiently widely realised, at all events in England. I note that an authority on the use of pneumococcal vaccine in the treatment of Pneumonia asserts,[8] in a paper which appeared some eighteen months after the publication of the discoveries to which I now refer—". . . differences in strain chiefly concern the virulence, and if a stock vaccine be made from a highly virulent strain this should be efficacious against infections caused by pneumococci of less virulence . . . for some time I have used one derived from a rapidly fatal case of pneumococcic Meningitis." I have seen no statement which even calls into question this assertion, far less any which definitely contradicts it. Until pneumococcal vaccines are

prepared in a rational manner, and in the light of our present knowledge, no progress can be expected either in the prophylaxis or in the treatment of Lobar Pneumonia by these means.

Investigation of Prevalent Groups of Pneumococci on the Rand.

As a preliminary to the study of prophylactic inoculation against Lobar Pneumonia it was necessary to secure a number of cultures of the organism from cases of Lobar Pneumonia at widely separated places on the Rand; by such means one could ascertain which were the more prevalent groups, and against how many of them it would be requisite to establish an immunity. Every group encountered should, theoretically, have been included in the experimental vaccine, but for practical reasons I deliberately excluded certain groups which collectively formed about 17% of the total investigated. Groups "A," "C," "D," "F," and "G" were employed throughout the experimental work; Group "B" was omitted because it happened to be relatively scarce when this particular investigation was initiated (1915).*

Table I. sets forth these groups, designates each by its group-letter, and records the numerical prevalence of each. The Table also records some observations as to the relative prevalence of different strains in 1913 and 1914. Every culture recorded in the Table was obtained by means of lung-puncture or blood-culture.

Very many experiments have been carried out with post-critical serum and the homologous organism and have amply confirmed the frequency and high degree of agglutination and phagocytosis previously described as occurring under these conditions.[4]

That the Pneumococcus is the causal organism in practically every case of Lobar Pneumonia amongst Natives on the Rand is borne out by the fact that in every case in which I have obtained a culture it has proved, without exception, to be the Pneumococcus—I include in this category two cultures of Pneumococcus mucosus; this statement refers to considerably more than 100 pneumonic cultures. It is true that I have lung-punctured about 100 other cases of suspected Lobar Pneumonia without obtaining a culture of any organism; as, however, the lung-puncture was on no occasion repeated on the same patient it is probable that I failed to obtain Pneumococci from many cases in which, in point of fact, they were present in the lung; it happens fairly frequently, moreover, that the puncture is a "dry" one. As against this failure to secure Pneumococci by these means in about 50% of my cases I have, on several occasions, obtained the Pneumococci by blood-culture when the lung-puncture, performed at the same time, gave a negative result. Notwithstanding this fact, however, I obtained far more cultures by lung-puncture than by blood-culture.

* Experiments more recently carried out by me show that the *specific* antibody-production elicited by a Group "B" vaccine is as satisfactory as that which occurs after the inoculation of a vaccine composed of members of the Groups "A," "C," "D," "F," and "G."

The Antibodies Selected for the Purpose of Gauging the Immunity-response in Inoculated Animals (including Man).

In previous investigations[1] I had satisfied myself that pneumococcal agglutinins and opsonins were present in the post-critical sera of at least 90% of patients recovering from Lobar Pneumonia. I found that these bodies could usually be first demonstrated at the time of the crisis, and occasionally twelve to twenty-four hours before the temperature had fallen to normal; I also found that they failed to appear in those cases in which no crisis occurred and which ended fatally. It is not an unwarranted assumption, therefore, that these two antibodies play an essential part in the biochemical changes which bring about the destruction of Pneumococci in Lobar Pneumonia, and which lead, in successful cases, to the complete recovery of the patient.

Agglutinins have been considered by the majority of workers as playing no important part, either directly or indirectly, in the destruction of bacteria; that their presence, associated as it usually is with that of other antibodies, frequently indicates a state of immunity is, however, generally conceded. There have been others who, on the other hand, have maintained that agglutinins play an active part against micro-organisms. Quite recently Bull[9] has shown by experiments (in which he treated pneumococcal septicaemia with homologous antisera) that agglutinins are of prime importance in the combating of pneumococcal infection in the rabbit. His conclusion is, that "Agglutination is not merely an incidental phenomenon but constitutes an essential process in association with phagocytosis in the protection of the rabbit against pneumococcus infection." He further states—"So far as the blood phenomena are concerned—the abrupt disappearance of the cocci from the circulating blood, agglutination and phagocytosis—active and passive immunity are similar."

Phagocytosis is far more generally recognised as a powerful factor in immunity against the Pneumococcus, and it can be almost constantly demonstrated with the post-critical blood serum from cases of Lobar Pneumonia provided that the homologous organism be used in the test.

Clough[10] regards the phagocytosis which is brought about by immune serum as the direct cause of the protective power of the latter. Bull, as already quoted, assigns a prominent position to phagocytosis. R. Cole[11] reaches the conclusion that leucocytes are a factor in preventing the local spread of the disease, and probably also in combating the general infection. Hektoen[12] holds that "The cure of Pneumonia results from the destruction of the Pneumococcus in the lungs and in the blood. This is accomplished by phagocytosis and also by extracellular digestive processes. The predominating general defensive reactions in Pneumonia are leucocytosis and the production of antibodies for Pneumococci, of which the opsonins are best known; and these appear to be specific for the group to which the infecting Pneumococcus belongs." Clough,[13] still more recently, discusses the method of destruction of the

Pneumococcus in Lobar Pneumonia and says—"The evidence too is strongly in favour of the view that this destruction is the result of phagocytosis and intracellular digestion of the organisms."

There is, then, a sufficient consensus of opinion, based upon experimental investigation, to justify my selection of these two antibodies as the criteria by which the progress of attempts at immunisation may be guided and perhaps evaluated. Similar principles were used in the well-known initial investigations in prophylactic anti-typhoid inoculation; in that case, however, other antibodies (notably bactericidins) were scrutinised in addition to the two now under consideration. It is worthy of remark, in passing, that bactericidins do not appear to be produced during the establishing of immuity against the Pneumococcus.

When speaking of phagocytosis I use the term in its widest sense, and although my observations have been directed solely to the polymorphonuclear leucocytes I do not conceive that the phenomenon of phagocytosis is necessarily limited to the leucocytes alone. Nor am I concerned with the question whether the antibodies which prepare the Pneumococcus for phagocytosis are "tropins," in the sense that Neufeld conveys, or "opsonins," in that of Sir Almroth Wright. Further, whatever importance is to be placed upon the presence of agglutinins and opsonins in the sera of inoculated animals (including man) I strongly suspect that there are other factors concerned in immunity against the Pneumococcus.

Methods used to Estimate the Amount of Antibody-production in the Serum of Inoculated Animals (including Man).

The technique I have employed to ascertain the agglutinating and opsonizing power of the sera of my inoculated animals (including man) has been essentially that originated by Sir Almroth Wright[14] in connection with his work on the opsonic index. I find this method capable of being used for the simultaneous estimation both of the phagocytosis and the agglutination brought about in phagocytic mixtures. I have, however, introduced one variation, viz., the use, at times, of more than one volume of the serum to be tested. It is usual in making phagocytic mixtures, according to Sir Almroth Wright's technique, to take up in the capillary glass tube one volume each of washed blood corpuscles, serum, and emulsion of micro-organisms; I found, however, when my work had been in progress a short while, that when a serum obtained from an individual during the course of immunisation failed to agglutinate the bacteria with the usual allowance of equal volumes of the constituents, that it nevertheless would often bring about complete agglutination when two, or sometimes three, volumes of serum were employed instead of one. This result depended upon the fact that the agglutinating titre of the serum under investigation was slightly below that necessary to be effective when only one volume was used. The degree of phagocytosis was affected in a similar manner.

It is, of course, well known that the degree of agglutination and of phagocytosis which occurs in the presence of any sample of serum *in vitro* may be influenced not only by the titre of the serum but also by the numerical richness of the emulsion of bacteria employed in the test. It may therefore be very naturally objected that the positive result which may be obtained when a greater proportion of serum is used may be entirely due to the fact that the degree of concentration of organisms in the phagocytic mixture has been reduced. That this is not the determining factor in this instance can be readily shown by ensuring that the concentration of the bacteria in the "unconventional" phagocytic mixtures is approximately equal to that in the usual mixture.

Normal human serum, when used as a control, will not agglutinate Pneumococci even when its proportion is 2, 3, 4, or even 5 times that of each of the other constituents. Recently isolated strains of Pneumococci which belong to any of the groups enumerated in Table I. are not, in fact, agglutinated by normal rabbit or human serum under the most favourable circumstances; the number of volumes of serum may be increased and the incubation period may be extended even to two hours, as against the usual fifteen minutes, without apparent effect.

It is worthy of remark that the degree of phagocytosis brought about by normal serum, under the most favourable circumstances of concentration, and provided that the culture used is of sufficiently recent isolation, is negligible as compared with the result obtained with the post-critical serum of a pneumonic patient, or the serum of a normal man or rabbit suitably immunised. With normal serum the phagocytic count of 50 leucocytes will rarely reach 20, whereas with the varieties of serum just referred to it will usually amount to more than 500, and will often exceed 3,000.

It is very necessary to insist upon the fact that normal serum, even when undiluted, will not agglutinate Pneumococci belonging to any of my groups. The method I used for classifying Pneumococci in 1913 was applied, in 1915, to the identification of Meningococci by Warren Crowe,[15] and in this latter instance some criticism has been directed against it. I am not in a position to discuss, from personal experience, the merits of this method when Meningococci are concerned, but on *a priori* grounds I think that a reliable technique is possible. When applied to the Pneumococcus the method, as serological tests go, is unusually reliable, for I know of no such test which gives accurate results in 100% of cases. In the present instance, however, I believe that the serological test will correctly identify members of any group enumerated in Table I. (down to Group "X") with an accuracy which is approximately one of 100%.

Estimations of the agglutinin content of serum, in the study of prophylactic immunity against the Pneumococcus, do not lend themselves to graphic methods of expression, *e.g.* by means of charted curves: the agglutination titre is usually too low, unless one works with a serum

which has been obtained by inoculating an animal with dead and living cultures over a lengthy period. Adequate information of the rise of agglutinating power can nevertheless be gained by employing respectively 3, 2, or 1 volumes of serum in a phagocytic mixture, spreading a drop of the mixture on slides, staining and examining them microscopically (as is done in estimating the opsonic index), and stating the degree of agglutination thus disclosed in terms of a conventional standard to be decided upon by the observer. A further advantage attending the use of this simple method lies in the fact that the opsonic quality of the serum may be simultaneously estimated from the same slides.

The difficulty of recording the result of opsonic estimations by means of charted curves is even greater than in the case of agglutination estimations. Normal serum will often give results that would have to be registered on the base line, whereas the opsonic capacity of the immune serum would appear as a line rising steeply to a point at the top of the chart: in such circumstances the steep curve would not truly represent the rise of the opsonic index, for no opsonic index can be computed when the normal serum affords no phagocytosis; on other occasions the normal serum might give a phagocytic count of, say, 10, whilst that of the immune serum would be, perhaps, 1,500, and it would not seem very informative to speak of an opsonic index of 150!

It may be objected that phagocytosis cannot be accurately estimated in a phagocytic mixture in which agglutination is also evident. The objection is not, however, well founded, as may be demonstrated when one makes use of an antiserum which, although it agglutinates powerfully has nevertheless, through lapse of time, lost its capacity of giving rise to phagocytosis; in such a case, although the cocci are fully agglutinated and drawn with the leucocytes to one end of the slide, none of the appearances of phagocytosis will be observed; the leucocytes are apparently pushed aside by the masses of agglutinated cocci and neither overlie nor underlie them.

It is appropriate for me to here draw attention to a statement made by Chickering[16] with reference to the serum of pneumonic patients; it is as follows—"The tests were made with serum as fresh as possible, for it has been found that agglutinins in human blood disappear quickly after it is stored. . . . With highly immune horse serum this is not the case." My experience is quite at variance with this, for I have found that post-critical pneumonic serum retains its agglutinating power for very long periods. I have specimens of such serum in my possession which are more than two years old, and which still give complete agglutination with organisms of their homologous group (using the technique already described). I consider this survival of agglutinins in post-critical human serum, stored *in vitro*, to be the rule and not the exception.

In the experiments which I here record I tested the sera of the various inoculated animals (including man) *separately*, and purposely refrained from making use either of pooled sera, or of phagocytic

mixtures which had been pooled before or after incubation. If the system of pooling sera had been adopted very serious fallacies would have arisen; it is obvious that if one sample of agglutinating serum be pooled with five samples of sera which do not agglutinate the positive result which may be obtained would be wrongly attributed to five out of the six contributors.

When recording the degree of agglutination brought about by a given serum I have stated, at the top of each Table, the number of volumes of serum employed in each test, and have noted the result by the usual signs of "—," "trace," "+," "++," and "+++"; the last sign ("+++") indicates complete agglutination.

The degree of phagocytosis observed is recorded side by side with that of agglutination, and the number of volumes of serum which is stated at the top of the Table applies equally to both tests. The contents of 25 leucocytes were enumerated in all cases in which this could be reasonably carried out. When, however, the count was so great that the time employed appeared to be in no way commensurate with the information to be gleaned the results are not recorded numerically but by the use of the following terms and signs:—"Uncountable" (*i.e.*, approximately 400 cocci in 25 leucocytes); "Uncountable +" (*i.e.*, approximately 600); "Uncountable ++" (*i.e.*, approximately 1,000); "Uncountable +++" (*i.e.*, estimate at more than 1,500).

I have found that when the serum of normal rabbits is under investigation the degree of phagocytosis is usually low, even with strains of Pneumococci which have been subcultured many times and which give an uncountable phagocytosis with normal human serum. The great superiority in this respect of the sera of immunised rabbits over that of normal rabbits is very apparent. Where human serum is concerned, however, *unless fresh cultures are frequently isolated and these alone used for the tests*, the normal controls will phagocyte most strains of Pneumococci so readily that the relative superiority of the sera from immunised individuals is less evident. Strain 3,472, a member of Group "C," has proved an exception to this rule, however, and after ten months' subculturing on blood-agar has still rendered obvious the contrast in opsonizing power between normal and immune human serum. Such a capacity for resisting phagocytosis is, however, not found amongst all strains belonging to this group.

As the difficulty which arises from the loss by the Pneumococcus of its resistance to phagocytosis on repeated subculture does not apply to agglutination I regard an observation of the latter phenomenon as the most practical and reliable method of evaluating the results of inoculation with this organism.

It will be seen in the subsequent records that I have rarely fulfilled the ideal condition stated above, viz., that of invariably using for the tests strains of Pneumococci which have been recently isolated and which are consequently resistant to phagocytosis in the presence of normal

human serum; nevertheless, from the results of many hundreds of tests carried out at different times when this essential condition was complied with, I am able to speak with confidence as to the validity of the recommendation.

Inoculation of Rabbits with Killed Pneumococcal Vaccine.

Comparison of the Intravenous and Subcutaneous Methods.

Detailed Consideration of Results of Intravenous Inoculations with Single-strain and Composite Vaccines.

The rabbit was selected for my initial work on account of the ease with which it can be intravenously inoculated, and because of its susceptibility to pneumococcal infection.

Previous experience had taught me that pneumococcal agglutinins and opsonins are present in the serum of rabbits which have been inoculated intravenously on several occasions with emulsions of *living* Pneumococci. Such procedure, however, would be impracticable when prophylaxis in human beings was the object to be attained, for it must be borne in mind that, for practical purposes, the vaccine must be a killed one and the number of inoculations must be limited—not exceeding, if possible, three in number.

With these practical considerations in view I took five rabbits, and after a preliminary testing of their sera by the method described (using 3, 2, and 1 volumes) I inoculated four of them on three occasions with killed Pneumococci; two received 100 millions—one intravenously and one subcutaneously, and two 400 millions—one intravenously and one subcutaneously. The fifth animal was set aside as an uninoculated control. The intervals between the first and second, and between the second and third, inoculations were five days and seven days respectively.

The vaccine used in this experiment was made by washing off 18-hour growth on blood-agar with normal saline, heating to 56°C. for one hour, and then adding enough carbolic acid to represent ·5% of the finished product. Cultures of strains 3472 and 3545, both members of Group "C," constituted the vaccine. The enumeration of organisms in this and all other vaccines employed throughout this study was carried out by Sir Almroth Wright's blood-corpuscle method.

I deemed it advisable to commence my investigations by endeavouring to immunise against one group only; in the event of this being successful I proposed to attempt a simultaneous immunisation against five groups.

TABLE II.

Inoculation of Rabbits No. 1, 2, 3, and 4 with a Vaccine prepared from Strains 3472 and 3545 (both of Group "C"). Dosage, Method of Administration, and Date.

Rabbit.	Weight in grms.	(1st Inoculation) Nov. 10th.	(2nd Inoculation) Nov. 15th.	(3rd inoculation) Nov. 22nd.
No. 1	1920	100 M. intravenously	100 M. intravenously	100 M. intravenously
No. 2	1560	100 M. subcutaneously	100 M. subcutaneously	100 M. subcutaneously
No. 3	1300	400 M. intravenously	400 M. intravenously	400 M. intravenously
No. 4	1530	400 M. subcutaneously	400 M. subcutaneously	400 M. subcutaneously

The sera of these four rabbits, and that of the one control animal, were tested, as already stated, before the experiment began (Table III.) and also at frequent intervals during its progress. Representative results are recorded below (Tables IV., V., and VI.).

For the sake of brevity and clearness no mention is made in these tables of the one volume of washed blood-corpuscles and the one volume of suspension of Pneumococci which were, of course, constituents of each phagocytic mixture. The corpuscles were derived from a "normal" laboratory worker (a European, "A.G.L."), the blood being collected in a 1·5% solution of sodium citrate, and washed twice in normal saline. The Pneumococci were always suspended in ·85% saline solution.

TABLE III.

(1, 2, and 3 Vols .Serum.)

Sera of Rabbits No. 1, 2, 3, and 4 tested against Strains 3472 and 3545, immediately before Inoculation with a Vaccine prepared from Cultures of these Strains.

Strain.	Serum.	(1 vol. Serum).		(2 vols. Serum.)		(3 vols. Serum).	
		No. of Cocci in 25 Leucocytes	Degree of Agglutination	No. of Cocci in 25 Leucocytes	Degree of Agglutination	No. of Cocci in 25 Leucocytes	Degree of Agglutination
3472 ("C")	No. 1	0	—	0	—	0	—
,,	No. 2	0	—	0	—	0	—
,,	No. 3	0	—	0	—	0	—
,,	No. 4	0	—	0	—	0	—
,,	No. 5 (Control)	0	—	2	—	0	—
3545 ("C")	No. 1	4	—	1	—	1	—
,,	No. 2	1	—	4	—	2	—
,,	No. 3	5	—	2	—	6	—
,,	No. 4	5	—	7	—	5	—
,,	No. 5 (Control)	0	—	2	—	0	—

It will be observed that, with the sera of these uninoculated animals, strain 3545 shows more susceptibility to phagocytosis than strain 3472; the amount of the phagocytosis with the former culture is, however, insignificant and there is no evidence of agglutination with either culture.

To set out the records of all the tests—which were carried out almost daily—would be too tedious, and I will record here only those results which were noted immediately before each reinoculation, together with those obtained on the seventh day following the third inoculation.

TABLE IV.

(3 Vols. Serum.)

Sera of Rabbits No. 1, 2, 3, and 4 tested against Strains 3472 and 3545, five days after the First Inoculation with a Vaccine prepared from Cultures of these Strains.

Strain.	Serum.	No. of Cocci in 25 Leucocytes.	Degree of Agglutination.
3472 ("C")	No. 1*	0	—
,,	No. 2†	0	—
,,	No. 3*	0	—
,,	No. 4†	0	—
,,	No. 5 (Control)	0	—
3545 ("C")	No. 1*	0	—
,,	No. 2†	1	—
,,	No. 3*	124	+ +
,,	No. 4†	0	—
,,	No. 5 (Control)	0	—

*Intravenously inoculated. † Subcutaneously inoculated.

TABLE V.

(1 Vol. Serum.)

Sera of Rabbits No. 1, 2, 3, and 4 tested against Strains 3472 and 3545, seven days after the Second Inoculation with a Vaccine prepared from Cultures of these Strains.

Strain.	Serum.	No. of Cocci in 25 Leucocytes.	Degree of Agglutination.
3472 ("C")	No. 1*	54	+ + +
,,	No. 2†	0	—
,,	No. 3*	41	+ + +
,,	No. 4†	0	—
,,	No. 5 (Control)	0	—
3545 ("C")	No. 1*	Uncountable.	+ + +
,,	No. 2†	0	—
,,	No. 3*	Uncountable.	+ + +
,,	No. 4†	17	—
,,	No. 5 (Control)	4	—

* Intravenously inoculated. † Subcutaneously inoculated.

TABLE VI.

(1 Vol. Serum.)

Sera of Rabbits No. 1, 2, 3, and 4 tested against Strains 3472 and 3545, seven days after the Third Inoculation with a Vaccine prepared from Cultures of these Strains.

Strain.	Serum.	No. of Cocci in 25 Leucocytes.	Degree of Agglutination.
3472 ("C")	No. 1*	Uncountable.	+ + +
,,	No. 2†	0	—
,,	No. 3*	Uncountable.	+ + +
,,	No. 4†	0	—
,,	No. 5 (Control)	0	—
3545 ("C")	No. 1*	Uncountable + +	+ + +
,,	No. 2†	8	—
,,	No. 3*	Uncountable + +	+ + +
,,	No. 4†	20	—
,,	No. 5 (Control)	10	—

* Intravenously inoculated. † Subcutaneously inoculated.

It is evident from these tabulated results that rabbits No. 1 and 3, which were inoculated intravenously, elaborated in their blood both agglutinins and opsonins in substantial quantities, whereas rabbits No. 2 and 4, which received subcutaneous inoculations, completely failed in the matter of agglutinins, and, as regards opsonins, that the slight rise above the control in the case of No. 4, with respect to strain 3545, is quite insignificant.

The rise in agglutinating power was very marked after the second inoculation, whilst the opsonins did not appear to become so markedly increased until after the third. In subsequent experiments I repeatedly succeeded in demonstrating agglutinins in the sera of rabbits on the fourth day following the first inoculation, and I have reached the conclusion that there are few, if any, exceptions to this rule—provided that a sufficiently large dose of vaccine has been inoculated intravenously.

The optimum intravenous dose for producing immunity in the rabbit, presuming that immunity against one group only is sought, is, I think, one of 800 millions, repeated on three occasions.

The formation of opsonins in the sera of intravenously inoculated rabbits does not occur either so early or with quite the same regularity as does that of agglutinins; a substantial increase of these substances has, however, been found in every case tested.

It is to be noted that there is some difference in the degree of both agglutination and phagocytosis when the same serum was employed with different strains, notwithstanding that the latter belonged to the same group. Table IV. shows that the serum of rabbit No. 3, after only one inoculation, agglutinated and opsonised strain 3545 but not 3472: and in Table V. we find that the serum of rabbits No. 1 and 3 opsonised strain 3545 to a greater extent than 3472. Neither of these strains, at the time of their isolation, were phagocyted in the presence of either normal rabbit or normal human serum; after subcultivation on blood-agar, however, No. 3545 rapidly lost its power of resistance, whilst No. 3472 retained it for 10 months when tested against normal human serum—which is constantly more potent than normal rabbit serum in this respect.

The greatest degree of agglutination and phagocytosis which is produced by the serum of an inoculated rabbit is not necessarily evidenced against the homologous vaccine organism; this fact is clearly illustrated in Tables No. IX., X., and XI.

The following Tables set out the reactions obtained with the sera of five rabbits before and after they had been inoculated (intravenously) with different doses of a pneumococcal vaccine, prepared in the manner already described, from strain 3472 alone. They were tested against this strain and also against two other strains (3545 and 737) belonging to the same group ("C"). Rabbit No. 5 was again used as a control, and it will be observed that strain No. 3545 had now become definitely susceptible to phagocytosis in the presence of the serum of this normal rabbit (*cf.* Tables V. and VI. with VII., IX., X., and XI.).

TABLE VII.

(3 Vols. Serum.)

Sera of Rabbits No. 22, 23, 24, 25, and 26 tested against Strains 3545, 3472 and 737 (all members of Group "C") previous to Intravenous Inoculation with a Vaccine prepared from Strain 3472.

Strain.	Serum.	No. of Cocci in 25 Leucocytes.	Degree of Agglutination.
3545 ("C")	Rabbit No. 22	34	—
,,	,, No. 23	36	—
,,	,, No. 24	65	—
,,	,, No. 25	54	—
,,	,, No. 26	59	—
,,	,, No. 5 (Control)	36	—
3472 ("C")	,, No. 22	0	—
,,	,, No. 23	0	—
,,	,, No. 24	0	—
,,	,, No. 25	2	—
,,	,, No. 26	0	—
,,	,, No. 5 (Control)	0	—
737 ("C")	,, No. 22	0	—
,,	,, No. 23	0	—
,,	,, No. 24	2	—
,,	,, No. 25	1	—
,,	,, No. 26	0	—
,,	,, No. 5 (Control)	0	—

The observations recorded above show that neither of the three strains were *agglutinated* by the serum of any of these uninoculated rabbits. Strain 3545 was, however, susceptible of phagocytosis in the presence of each of these sera, whilst 3472 and 737 were insusceptible in the presence of any of them, although three volumes of sera were used in every instance. After this test had been completed, and on the same day, rabbits No. 22, 23, 24, 25, and 26 were inoculated, as follows, with a vaccine prepared from a culture of strain 3472 alone. Three inoculations were given intravenously to each rabbit; the interval between the first and second was four days, that between the second and third five days.

TABLE VIII.

Intravenous Inoculation of Rabbits No. 22, 23, 24, 25, and 26 with a Vaccine prepared from Strain 3472 (Group "C"), Dosage, and Dates.

	Weight.	Jan. 18th. First Inoculation.	Jan. 22nd. 2nd Inoculation.	Jan. 27th. 3rd Inoculation.
Rabbit 22	1600 grms.	50 millions	50 millions	50 millions
,, 23	1480 ,,	100 ,,	100 ,,	100 ,,
,, 24	1510 ,,	400 ,,	400 ,,	400 ,,
,, 25	1450 ,,	800 ,,	800 ,,	800 ,,
,, 26	1230 ,,	2000 ,,	2000 ,,	2000 ,,

TABLE IX.

(3 Vols. Serum.)

Sera of Rabbits No. 22, 23, 24, 25, and 26 tested against Strains 3545, 3472, and 737 (all members of Group "C"), four days after the First Intravenous Inoculation with a Vaccine prepared from a Culture of Strain 3472.

Strain.	Serum.	No. of Cocci in 25 Leucocytes.	Degree of Agglutination.
3545 ("C")	Rabbit No. 22	130	+ +
,,	,, No. 23	93	+ + +
,,	,, No. 24	Uncountable	+ +
,,	,, No. 25	Uncountable	+ + +
,,	,, No. 26	Uncountable	+ + +
,,	,, No. 5 (Control)	29	—
3472 ("C")	,, No. 22	0	—
,,	,, No. 23	0	—
,,	,, No. 24	0	—
,,	,, No. 25	0	+
,,	,, No. 26	0	+
,,	,, No. 5 (Control)	0	—
737 ("C")	,, No. 22	0	—
,,	,, No. 23	0	—
,,	,, No. 24	0	—
,,	,, No. 25	2	—
,,	,, No. 26	0	—
,,	,, No. 5 (Control)	0	—

The results recorded in this table demonstrate a differential elaboration of agglutinins and opsonins, with respect to three strains of Group "C," in the sera of rabbits four days after one inoculation with various doses of the same vaccine.

It is to be especially noted that the best results, not only as regards phagocytosis but also agglutination, were obtained with a strain (3545) other than that used for the preparation of the vaccine.

The next Table (No. X.) records the effects produced after two inoculations; the test was performed on the fifth day following the second inoculation.

TABLE X.

(1 Vol. Serum.)

Sera of Rabbits No. 22, 23, 24, 25, and 26 tested against Strains 3545, 3472, and 737 (all members of Group "C"), five days after the Second Intravenous Inoculation with a Vaccine prepared from a Culture of Strain 3472.

Strain.	Serum.	No. of Cocci in 25 Leucocytes.	Degree of Agglutination.
3545 ("C")	Rabbit No. 22	Uncountable	+ + +
,,	,, No. 23	,,	+ + +
,,	,, No. 24	,,	+ + +
,,	,, No. 25	,,	+ + +
,,	,, No. 26	,,	+ + +
,,	,, No. 5 (Control)	34	—
3472 ("C")	,, No. 22	10	—
,,	,, No. 23	40	+ +
,,	,, No. 24	44	+ +
,,	,, No. 25	Uncountable	+ + +
,,	,, No. 26	214	+ +
,,	,, No. 5 (Control)	0	—
737 ("C")	,, No. 22	6	—
,,	,, No. 23	5	Trace
,,	,, No. 24	9	+
,,	,, No. 25	Uncountable	+ +
,,	,, No. 26	5	+
,,	,, No. 5 (Control)	0	—

From Table No. X., where the titre of the various sera had increased sufficiently to allow of my using only *one* volume, we see that the second inoculation had elicited a far more general agglutinating power in the various sera (No. 22, the recipient of the smallest dose, excepted) and that all the strains now fell within their influence. The rise in opsonins had progressed in a similar manner but to a lesser degree.

The next Table (No. XI.) demonstrates the condition produced by the third inoculation; the test was performed six days after this event.

TABLE XI.

(1 Vol. Serum.)

Sera of Rabbits No. 22, 23, 24, 25, and 26 tested against Strains 3545, 3472, and 737 (all members of Group "C"), six days after the Third Intravenous Inoculation with a Vaccine prepared from a Culture of Strain 3472.

Strain.	Serum.	No. of Cocci in 25 Leucocytes.	Degree of Agglutination.
3545 ("C")	Rabbit No. 22	Uncountable	+ + +
,,	,, No. 23	,,	+ + +
,,	,, No. 24	,, + + +	+ + +
,,	,, No. 25	,, + + +	+ + +
,,	,, No. 26	,, +	+ +
,,	,, No. 5 (Control)	33	—
3472 ("C")	,, No. 22	70	Trace
,,	,, No. 23	240	+ + +
,,	,, No. 24	Uncountable	+ + +
,,	,, No. 25	,, + + +	+ + +
,,	,, No. 26	,, + +	+ + +
,,	,, No. 5 (Control)	0	—
737 ("C")	,, No. 22	0	—
,,	,, No. 23	119	+
,,	,, No. 24	Uncountable	+ + +
,,	,, No. 25	,,	+ + +
,,	,, No. 26	,, + + +	+ + +
,,	,, No. 5 (Control)	0	—

This table discloses a further increase in the titre of some of the sera, both as regards agglutinins and opsonins, particularly where the more resistant strains, 3472 and 737, were in question. This again exemplifies the fact that the vaccine organism may be found to be more resistant to the antibodies which it has given rise to than are other members of the same group.

Vaccines prepared from members of Group "C" were used to inoculate, by the intravenous method, 18 rabbits in all. The results were in every case similar to those just recorded, with the reservation that there was evidence that some members of this group provided better antigens than others. As an antigen, I came to regard strain 3472 as the best out of four which I investigated; the differences were, however, those of degree only, for in each instance agglutinins and opsonins were elicited in the sera of the experimental rabbits.

The experiments above recorded emphasize the degree of accuracy with which the antibodies under review can be evaluated; graduated results may be obtained, not only by using 3, 2, and 1 volumes of the sera, but also by employing test strains whose differences in resistance, both to agglutination and opsonification, have been previously determined, and which, more especially with respect to their agglutinability, remain constant through long periods.

A similar series of experiments, using a vaccine prepared from members of Group "A" was carried out and the results were in each instance similar to those already described.

A point which had now to be determined was the capacity possessed by the rabbit to elaborate, simultaneously, agglutinins and opsonins specific for each of five groups of the Pneumococcus when the animal was inoculated with a vaccine comprising all the groups.

Such a vaccine was prepared and numerically standardized by the methods already described as having been employed for the Group "C" vaccine. The groups represented in the vaccine were "A," "C," "D," "F," and "G," and 1 c.c. of the finished product contained 2,000 million cocci of each group, *i.e.* a total strength of 10,000 million cocci per c.c. Three rabbits, No. 35, 36, and 37, were inoculated at the times and in the manner shown in Table XII.

TABLE XII.

Intravenous Inoculation of Rabbits No. 35, 36, and 37 with a Vaccine composed of Pneumococci of Groups "A," "C," "D," "F," and "G." Dosage, and Dates.

	Weight.	March 29th. 1st Inoculation.	April 6th. 2nd Inoculation.	April 13th. 3rd Inoculation.
Rabbit 35	2,800 grms.	20,000 millions	20,000 millions	20,000 millions
„ 36	2,520 „	1,000 „	1,000 „	1,000 „
„ 37	2,410 „	2,000 „	2,000 „	2,000 „

To arrive at the number of Pneumococci of any one group inoculated on any single occasion it is only necessary to divide the total by five.

My object in administering so large a dose as twenty thousand million cocci intravenously was to ascertain, roughly, whether such a dose was toxic, and also to discover whether a negative phase would be produced, and if so whether it would delay or completely inhibit the formation of antibodies.

For the sake of brevity I shall record only the final result obtained after three inoculations, but it may be stated that the elaboration of agglutinins and opsonins progressed in a manner similar to that in the animals referred to in previous tables.

Table No. XIII. records the final results obtained upon testing the sera of these three rabbits, together with the serum of a control rabbit, against each of five strains representing the five groups of Pneumococcus represented in the vaccine used. Although the doses which were employed in the cases of rabbits No. 36 and 37 were less than the dose which I consider to be the optimum the results were nevertheless satisfactory—even when only one volume of serum was used in the test. It must always be kept in mind that when one volume of serum affords such decided results a still greater degree of both agglutination and phagocytosis would have occurred if two or three volumes had been employed, whilst no such increase of results could be brought about by a similar increase of the control serum (*vide* Table XIV.).

TABLE XIII.

(1 Vol. Serum.)

Sera of Rabbits No. 35, 36, and 37 tested against Strains of Groups "A," "C," "D," "F," and "G," four days after the Third Intravenous Inoculation with a Vaccine made from Cultures of these Strains.

Strain.	Serum.	No. of Cocci in 25 Leucocytes.	Degree of Agglutination.
2480 ("A")	Rabbit No. 35	Uncountable	∓ ∓ ∓
,,	,, No. 36	,,	∓ ∓ ∓
,,	,, No. 37	,,	∓ ∓ ∓
,,	,, No. 38 (Control)	0	—
3172 ("C")	,, No. 35	Uncountable	+ + +
,,	,, No. 36	0	∓
,,	,, No. 37	0	Trace
,,	,, No. 38 (Control)	0	—
300 ("D")	,, No. 35	Uncountable	+ + +
,,	,, No. 36	,,	+ + +
,,	,, No. 37	,,	+ + +
,,	,, No. 38 (Control)	6	—
506 ("F")	,, No. 35	Uncountable	+ + +
,,	,, No. 36	,,	+ + +
,,	,, No. 37	,,	+ + +
,,	,, No. 38 (Control)	3	—
6 ("G")	,, No. 35	Uncountable	+ + +
,,	,, No. 36	,,	+ + +
,,	,, No. 37	,,	+ + +
,,	,, No. 38 (Control)	12	—

The results shown in the above table disclose satisfactory antibody-production to all five groups, with the exception that the two rabbits, No. 36 and 37, which received the smaller doses, reacted feebly to Group "C."

Rabbit No. 35 made the best response notwithstanding the massive doses administered (twenty thousand million cocci on three occasions). This latter fact affords some evidence that the pneumococcal vaccine, as prepared for these experiments, is at most but very feebly toxic, and that a prolonged negative phase is not caused by very large doses. This animal (No. 35) had elaborated agglutinins on the fourth day after its first inoculation, and no rabbit in any of my experiments has done so at an earlier date.

Table XIV. gives the results of a test performed, with two and three volumes of the serum of two of these animals, against the relatively resistant strain 3472; and in Table XV. are recorded the results of testing the sera of all three animals, using one volume, against a strain which had been repeatedly passed through mice.

TABLE XIV.

(2 and 3 Vols. Serum.)

Sera of Rabbits No. 36 and 37 tested against Strain 3472 (Group "C"), four days after the Third Intravenous Inoculation with a Vaccine comprising Pneumococci of Groups "A," "C," "D," "F," and "G," using 2 and 3 volumes of sera.

Strain.	Serum.	No. of Cocci in 25 Leucocytes.	Degree of Agglutination.
3472 ("C")	Rabbit No. 36 (2 vols.)	3	+ +
,,	,, No. 36 (3 vols.)	80	+ + +
,,	,, No. 37 (2 vols.)	2	+
,,	,, No. 37 (3 vols.)	17	+ +
,,	,, No. 38 (Control) (2 vols.)	0	—
,,	,, No. 38 (Control) (3 vols.)	0	—

The results recorded in the foregoing Table show the greater degree of agglutination which occurred when two, or three, volumes of the sera of the inoculated rabbits were used, as contrasted with the absence of agglutination with the serum of the control animal.

TABLE XV.

(1 Vol. Serum.)

Sera of Rabbits No. 35, 36, and 37 tested against Strain 3545 (Group "C"), after direct passage of this Strain through 11 Mice, four days after the Third Intravenous Inoculation with a Vaccine comprising Pneumococci of Groups "A," "C," "D," "F," and "G."

Strain.	Serum.	No. of Cocci in 25 Leucocytes.	Degree of Agglutination.
3545 ("C")	Rabbit No. 35	Uncountable	+ + +
,,	,, No. 36	19	+ + +
,,	,, No. 37	10	+ +
,,	,, No. 38 (Control)	0	—

The strain used in this experiment (Table XV.) was that numbered 3545—another member of Group "C" and the least refractory member of this group in my possession. The actual culture used was the first sub-culture on blood-agar from the heart-blood of the last of a series of eleven mice, ten of which had been directly inoculated with the infected blood of the preceding animal.

If the results in this table be compared with those previously obtained with the same strain, as recorded in Tables III., IV., V., VI., VII., IX., X., and XI., it will be seen that the passage of the strain through 11 mice had restored the degree of resistance to phagocytosis in the presence of normal rabbit serum which it originally possessed; this resistance, however, again rapidly disappeared after subculturing on blood-agar.

It may also be noted that this passage through mice had not materially affected the capacity of the strain to be agglutinated; this conclusion is suggested by a comparison of the results shown in Table No. XV. with those relating to Culture 3472 in Table No. XIII. The volumes of sera employed in these instances were the same, and the two cultures belonged to the same group, viz., "C."

Subcutaneous Inoculation of Rabbits with Large Doses of Vaccine.

I now attempted to secure an antibody-production, as marked as that obtained by intravenous inoculation, by the subcutaneous administration of large doses of vaccine, and for this purpose Strain 3472 (Group "C") was once more selected. Four rabbits were inoculated as follows:—

TABLE XVI.

Subcutaneous Inoculation of Rabbits No. 29, 30, 31, and 32 with a Vaccine prepared from a Culture of Strain 3472 (Group "C"). Dosage, and Dates.

	Weight.	Feb. 9th. 1st Inoculation.	Feb. 14th. 2nd Inoculation.	Feb. 21st. 3rd Inoculation.
Rabbit 29	1250 grms.	1000 millions	1000 millions	1000 millions
,, 30	1120 ,,	1500 ,,	1500 ,,	1500 ,,
,, 31	1190 ,,	2000 ,,	2000 ,,	2000 ,,
,, 32	1330 ,,	3000 ,,	3000 ,,	3000 ,,

Table No. XVII. shows the results obtained on testing the sera of these rabbits, together with that of a control rabbit, eight days after the third inoculation. The strains employed in this test were 3545 and 3472, both belonging to Group "C." One volume of serum was used.

TABLE XVII.

(1 Vol. Serum.)

Sera of Rabbits No. 29, 30, 31, and 32 tested against Strains 3545 and 3472 (both members of Group "C"), eight days after the Third Subcutaneous Inoculation with a Vaccine prepared from a Culture of Strain 3472.

Strain.	Serum.	No. of Cocci in 25 Leucocytes.	Degree of Agglutination.
3545 ("C")	Rabbit No. 29	Uncountable	+ + +
,,	,, No. 30	,,	+ + +
,,	,, No. 31	,,	+
,,	,, No. 32	,,	+ +
,,	,, No. 5 (Control)	150	—
3472 ("C")	,, No. 29	0	Trace
,,	,, No. 30	Uncountable	+ + +
,,	,, No. 31	0	—
,,	,, No. 32	Uncountable	+ + +
,,	,, No. 5 (Control)	0	—

It is evident from the above table that where strain 3545 is the test-culture the antibody-production was satisfactory. The control rabbit's serum, however, showed a marked degree of phagocytosis; this culture had not, at this time, been passed through a series of mice.

In the case of the refractory test-culture 3472 (which had been used as the antigen), the production of antibodies is seen to have been irregular; rabbits No. 29 and 31 failed rather pronouncedly, whereas No. 30 and 32 made a satisfactory immunising-response.

A test performed on the same day with the sera of rabbits 29 and 31, but using three volumes instead of one, demonstrated the presence in them of both agglutinins and opsonins for strain 3472; the control serum, in three volumes, gave quite negative results to the same strain.

After the great regularity in the production of agglutinins and opsonins that I had observed in the sera of rabbits which had undergone intravenous inoculations with far smaller doses, I was somewhat disappointed that the subcutaneous inoculations yielded less satisfactory results in spite of the large increase in dosage; this experiment served to prove, however, that three inoculations of a vaccine of killed Pneumococci, given subcutaneously and in suitable doses, are capable of giving rise to both agglutinins and opsonins in the sera of rabbits. Further experiments dealing with the subcutaneous inoculation of pneumococcal vaccine is reserved for the section dealing with inoculations in man, in which connection this procedure comes to occupy a position of prime importance.

The Protection Conferred on Rabbits against Pneumococcal Septicaemia by Previous Intravenous Inoculations of Vaccine.

Having established the fact that agglutinins and opsonins are constantly produced in the sera of rabbits which have been suitably inoculated with killed cultures of the Pneumococcus, it became desirable to ascertain by crucial experiment whether or not rabbits presenting these characteristics in their blood would survive an inoculation with several lethal doses of a living culture.

My original control rabbit (No. 5) died on January the 28th from an accidentally acquired Pneumococcal Septicaemia. The animal, presumably, had been infected in the laboratory on one of the numerous occasions when a sample of blood had been withdrawn from a vein in the ear. I identified the infecting organism as a member of Group "C," and by its characteristic behaviour (to which I have already referred) as regards agglutination and phagocytosis in the presence of sera from inoculated rabbits I recognised it as being probably strain 3472, or as one having identical characteristics. The culture from this animal seemed suitable for use in the protection experiment decided upon; it belonged, as already stated, to Group "C," and I had on hand a number of rabbits which had been inoculated with a vaccine corresponding to this group.

In order to obtain some idea of the virulence of this culture six normal rabbits were inoculated subcutaneously with a 24-hour broth culture in the doses and with the results recorded in Table XVIII.

TABLE XVIII.

Results of Subcutaneous Inoculation of six normal Rabbits with a living broth Culture of Pneumococcus (Group "C").

	Weight.	Date.	Dose.	Result.
Rabbit No. 39	1320 grms.	Feb. 1st (Noon)	100 millions	Died Feb. 6th (4 p.m.)
,, No. 40	1380 ,,	,, ,,	200 ,,	,, Feb. 4th (night)
,, No. 41	1500 ,,	,, ,,	400 ,,	,, Feb. 5th (5 p.m.)
,, No. 42	1450 ,,	,, ,,	800 ,,	,, Feb. 3rd (11p.m.)
,, No. 43	1420 ,,	,, ,,	1200 ,,	,, Feb. 3rd (12p.m.)
,, No. 44	1110 ,,	,, ,,	1600 ,,	,, Feb. 3rd (11 a.m.)

The above results indicated that the minimal lethal dose of this particular culture for normal rabbits, when inoculated subcutaneously, was probably less than 100 millions; in order to make the experiment which I shall now describe conclusive I therefore decided to employ a subcutaneous injection of 800 million organisms—at least eight times the M.L.D. At the time of the experiment the strain had probably become slightly more virulent as it had in the meantime been passed through another rabbit and the culture actually used was the first sub-culture on blood-agar from the heart-blood of this second animal.

A subcutaneous injection of 800 million organisms of this culture was administered to six normal rabbits, No. 45, 46, 47, 48, 49, and 50, and also to six rabbits that had previously received intravenous inoculations of pneumococcal vaccine (Group "C"), viz., No. 1, 22, 23, 24, 25, and 26 (see Tables II., VI., VIII., and XI.). In order to give every chance to the normal control rabbits I selected older and, with one exception, heavier animals. As all the inoculated rabbits employed in this experiment have figured in the Tables referred to the protecting doses which they had received, together with the condition of their sera as regards agglutinins and opsonins, can be ascertained.

TABLE XIX.

Results of subcutaneously inoculating with living Pneumococci (Group "C") six Rabbits which had been previously inoculated with killed Cultures (Group "C") and six control Rabbits.

	Weight.	Date.	Dose.	Result.
Rabbit No. 1 Inoculated	2170 grms.	Feb. 4th, 12 (noon)	800 millions	Survived
,, No. 22 ,,	1750 ,,	,, ,, ,,	,, ,,	,,
,, No. 23 ,,	1650 ,,	,, ,, ,,	,, ,,	,,
,, No. 24 ,,	1750 ,,	,, ,, ,,	,, ,,	,,
,, No. 25 ,,	1610 ,,	,, ,, ,,	,, ,,	,,
,, No. 26 ,,	1410 ,,	,, ,, ,,	,, ,,	,,
,, No. 45 Control	2150 ,,	,, ,, ,,	,, ,,	Died. Feb. 6th (Night)
,, No. 46 ,,	2470 ,,	,, ,, ,,	,, ,,	,, ,, 7th (7 a.m.)
,, No. 47 ,,	2600 ,,	,, ,, ,,	,, ,,	,, ,, 5th (Night)
,, No. 48 ,,	1900 ,,	,, ,, ,,	,, ,,	,, ,, 5th (Night)
,, No. 49 ,,	2070 ,,	,, ,, ,,	,, ,,	,, ,, 5th (Night)
,, No. 50 ,,	2600 ,,	,, ,, ,,	,, ,,	,, ,, 5th (Night)

Note. Pneumococci were recovered from the heart-blood of each rabbit that died.

These results appear to be conclusive; all the previously inoculated animals survived and all the normal controls died.

The appearance of usual health in the previously inoculated animals was very noticeable—they readily ate their food and seemed unaffected by the inoculum; not merely did they survive, they never appeared to be even ill.

On referring to Tables II., VI., VIII., and XI., in which the doses of vaccine administered and the results of serological tests in the case of these rabbits are stated, it is seen that the doses varied from 50 million to 2,000 million cocci, and that only one animal had received a dose of 800 millions, which I have previously stated to be in my opinion the optimum for a rabbit where the intravenous method is used.

From Table XI. it is to be observed that the serum of Rabbit No. 22, which had received three doses of only 50 million cocci, failed both to agglutinate and opsonise strain 737 (Group "C") and gave but a trace of agglutination and a relatively small degree of phagocytosis with strain 3472 (Group "C"); despite these facts the animal survived its test dose.

On another occasion I submitted another rabbit, No. 35 (see Tables XII. and XIII.) to a test inoculation and found that it survived what were probably ten lethal doses; the control in this instance received what was probably one lethal dose with a fatal result.

Taking all these facts into consideration one is justified, I think, in concluding that it is possible to immunise rabbits against many lethal doses of living Pneumococci by inoculating them intravenously on three occasions, at intervals of seven days, with suitable doses of a killed pneumococcal vaccine. It is even probable that even one intravenous dose of sufficient size affords some measure of protection since such an inoculation elicits the production of agglutinins and opsonins which can be demonstrated in the sera of animals so inoculated.

With regard to the protection conferred by subcutaneous inoculations—although I have not submitted rabbits inoculated in this manner to crucial protection-tests yet the presence in their sera of specific agglutinins and opsonins is evidence of the presence of a definite immunity in their case also. I regard the presence more particularly of agglutinins for the Pneumococcus in the serum of a rabbit as an indication that the animal possesses some degree of immunity against members of that group to which the agglutinated Pneumococcus belongs. When we remember that the susceptibility of Pneumococci to be agglutinated by an antiserum is a relatively constant phenomenon, as compared with their variable susceptibility to phagocytosis, it is

apparent that the phenomenon of agglutination is a more reliable guide in the evaluation of immunity than is that of phagocytosis.

It has not been deemed necessary for the purpose of this work to make use of absorption experiments. There is, with few exceptions, such positive evidence in favour of the power of any member of a given group of Pneumococci to elicit the formation of antibodies which are specific for all members of the group that further enquiry in this direction, having regard to the limitations of time at my disposal, seemed unwarranted. In the considerable amount of investigation by various workers which has been directed to this end the only exception I have seen recorded is that with respect to three cultures—which I have already referred to as having been placed by O. T. Avery[5] in sub-group "II.x," which corresponds to a sub-group of my Group "B."

Intravenous Inoculation of Pneumococcal Vaccine in Man.

In continuation of the logical sequence of these experiments I attempted to produce in the blood of man, by the inoculation of vaccine, the same characteristics—agglutinating and opsonising power—that I had succeeded in establishing in rabbits.

My own serum had been used as a normal control on many hundreds of occasions where the Pneumococcus was under investigation, not only during Sir Almroth Wright's work in Johannesburg, in 1911-1912, but throughout the three subsequent years; in no single instance had it agglutinated any strain of Pneumococcus and I therefore decided to use myself for the purpose of an experiment.

The following table, No. XX., records the dosage, time, and method of my self-inoculation. I used for this purpose the same batch of vaccine (prepared from strains 2480, 3472, 300, 506, and 6—Groups "A," "C," "D," "F," and "G") that had previously been employed in the experiments dealing with Rabbits 35, 36, and 37 (*vide* Tables XII. and XIII.). Each c.c. of this vaccine contained 10,000 million cocci, comprising 2,000 million of each group.

TABLE XX.

Intravenous Inoculation of myself with a composite Vaccine. Dosage and Dates.*

	Feb. 15th (1st Inoculation).	Feb. 22nd (2nd Inoculation).	Feb. 28th (3rd Inoculation).
F.S.L.	2,000 millions.	2,000 millions.	4,000 millions.

Neither the first nor the second inoculation gave rise to any production of agglutinin or opsonin in my blood, both antibodies were, however, demonstrable a few days after the third dose.

The results of testing my serum against the five groups inoculated are set out in the next table; the control was my European Laboratory Assistant.

TABLE XXI.

(3 Vols. Serum.)

Author's serum tested against Groups "A," "C," "D," "F," and "G," five days after the Third Intravenous Inoculation with a composite Vaccine.

Strain.	Serum.	No. of Cocci in 25 Leucocytes.	Degree of Agglutination.
2480 ("A")	F.S.L.	Uncountable	+ +
,,	A.G.L. (Control)	102	—
3472 ("C")	F.S.L.	Uncountable	+ +
,,	A.G.L. (Control)	60	—
300 ("D")	F.S.L.	Uncountable	Trace
,,	A.G.L. (Control)	,,	—
506 ("F")	F.S.L.	,,	—
,,	A.G.L. (Control)	66	—
6 ("G")	F.S.L.	Uncountable	+ + +
,,	A.G.L. (Control)	127	—

* The vein selected was the Median Basilic.

It is seen from the results recorded in this table that the Author's serum had elaborated agglutinins and opsonins in respect of four out of the five groups made use of. For the fifth group ("F") no agglutinins were demonstrable, but the opsonins were present in an amount far greater than in the normal control serum.

The antibody-content of my serum continued to increase without further inoculation, and Table No. XXII. gives the results of a test performed on April the 11th, six weeks after the third and final inoculation. On this occasion it was possible to demonstrate the presence of agglutinins by employing only one volume of serum.

TABLE XXII.

(1 Vol. Serum.)

Author's serum tested against Groups "A," "C," "D," "F," and "G," six weeks after the Third Intravenous Inoculation with a composite Vaccine.

Strain.	Serum.	No. of Cocci in 25 Leucocytes.	Degree of Agglutination.
2480 ("A")	F.S.L.	Uncountable + +	+ + +
,,	A.G.L. (Control)	136	—
3472 ("C")	F.S.L.	34	+
,,	A.G.L. (Control)	5	—
300 ("D")	F.S.L.	Uncountable + +	+
,,	A.G.L. (Control)	,,	—
506 ("F")	F.S.L.	,, +	—
,,	A.G.L. (Control)	98	—
6 ("G")	F.S.L.	Uncountable + +	+ + +
,,	A.G.L. (Control)	175	—

It is to be remarked that these results—employing only one volume of the serum—are equally as good (the reactions to strain 3472 excepted) as those recorded in Table XXI., where three volumes were used. On the same day I further tested the reaction between the relatively resistant strain 3472, my serum, and that of my control, making use of *two* volumes; under these conditions complete agglutination and uncountable phagocytosis occurred with my serum, whilst with that of my control (A.G.L.) the phagocytic count was 12 and agglutination was absent.

Three Natives from Portuguese East Africa were now intravenously inoculated with three doses, each of 2,000 millions, of the same vaccine as I had used for myself. The results obtained were similar to those described in my own case, with the exception that all three elaborated agglutinins to Group "F" in addition to those for the other four groups concerned.

Two East African Natives were then inoculated with larger doses of the same vaccine, as follows:—

TABLE XXIII.

Intravenous Inoculation of two East African Natives with a composite Vaccine. Dosage and Dates.

	March 8th (1st Inoculation).	March 15th (2nd Inoculation).	March 21st (3rd Inoculation).
Native No. 2377 (Shangaan)	4,000 millions	4,000 millions	4,000 millions
Native No. 2579 (M'chopi)	8,000 ,,	8,000 ,,	8,000 ,,

I give below (Table XXIV.) the results of testing these two sera and that from a European control (A.G.L.) six weeks after their third inoculation. It is not necessary to record in detail the results of serological tests made after the first and second inoculations; suffice it to state that traces of agglutination were demonstrated seven days after their first inoculation, that these had increased substantially on the sixth day after the second inoculation, and that six days after the third inoculation the degree of agglutination and phagocytosis of all five strains in the presence of both sera had reached a satisfactory degree—only one volume of serum being employed.

TABLE XXIV.

(1 Vol. Serum.)

Sera of Natives No. 2377 and 2579 tested against Groups "A," "C," "D," "F," and "G" six weeks after the Third Intravenous Inoculation with a composite Vaccine.

Strain.	Serum.	No. of Cocci in 25 Leucocytes.	Degree of Agglutination.
2480 ("A")	2377	Uncountable + + +	+ + +
,,	2579	,, + + +	+ + +
,,	A.G.L. (Control)	186	—
3472 ("C")	2377	Uncountable	+ + +
,,	2579	36	+ + +
,,	A.G.L. (Control)	13	—
300 ("D")	2377	Uncountable	+
,,	2579	,,	+ +
,,	A.G.L. (Control)	80	—
506 ("F")	2377	Uncountable + + +	+ + +
,,	2579	,, + + +	+ + +
,,	A.G.L. (Control)	89	—
6 ("G")	2377	Uncountable + + +	+ + +
,,	2579	,, + + +	+ + +
,,	A.G.L. (Control)	100	—

Neither of these Natives were prevented from carrying out their usual employment for a single day as a result of their inoculations. In view of these mild reactions I decided to inoculate three other East African Natives with still larger doses. Three healthy Natives were selected and a preliminary test was performed with their sera in respect to each of the five strains contained in the vaccine. Agglutination was absent in all cases. They were then inoculated with the vaccine as follows:—

TABLE XXV.

Intravenous Inoculation of three East African Natives with a composite Vaccine. Dosage and Dates.

	April 5th (1st Inoculation).	April 12th (2nd Inoculation).	April 19th (3rd Inoculation).
Native No. 1911	2,500 millions	5,000 millions	10,000 millions
,, No. 113	5,000 ,,	10,000 ,,	20,000 ,,
,, No. 871	10,000 ,,	20,000 ,,	40,000 ,,

The results obtained on testing the serum of a European control and the sera of these Natives, 13 days after their third inoculation, are set out in Table XXVI. Details of the tests performed during the course of the three inoculations are again omitted. It is to be remarked, however, that the serum of Native 871, who had received an initial dose of ten thousand million cocci, agglutinated and opsonised the Pneumococcus of all the groups, excepting "D," six days subsequent to the first inoculation.

TABLE XXVI.

(1 Vol. Serum.)

Sera of Natives No. 1911, 113, and 871 tested against Groups "A," "C," "D," "F," and "G," thirteen days after the Third Intravenous Inoculation with a composite Vaccine.

Strain.	Serum.	No. of Cocci in 25 Leucocytes.	Degree of Agglutination.
2480 ("A")	1911	Uncountable + + +	+ + +
,,	113	,, + + +	+ + +
,,	871	,, + + +	+ + +
,,	A.G.L. (Control)	101	—
3472 ("C")	1911	Uncountable + + +	+ + +
,,	113	,, + + +	+ + +
,,	871	,,	+ + +
,,	A.G.L. (Control)	16	—
300 ("D")	1911	Uncountable	—
,,	113	,,	Trace
,,	871	,,	+
,,	A.G.L. (Control)	200	—
506 ("F")	1911	Uncountable + + +	+ + +
,,	113	,, + + +	+ + +
,,	871	,, + + +	+ + +
,,	A.G.L. (Control)	100	—
6 ("G")	1911	Uncountable + + +	+ + +
,,	113	,, + + +	+ + +
,,	871	,, + + +	+ + +
,,	A.G.L. (Control)	125	—

The above table shows that remarkably good results, from the point of view of antibody-production, were obtained with the three Natives' sera, excepting in the case of "D" group. This group has been the least amenable in nearly all of the inoculations in man. I have previously referred to the fact that some strains possess better antigenic properties than others; unfortunately this was the only culture belonging to Group "D" which was available and it had been subcultured on blood-agar for more than eighteen months. Had I other strains of this group to select from I should probably have obtained more satisfactory results.

Subcutaneous Inoculation of Pneumococcal Vaccine in Man.

I have previously shown (Tables XVI. and XVII.) that agglutinins and opsonins were produced in the blood of rabbits which had received three suitable inoculations subcutaneously. Some two hundred thousand Native labourers are constantly employed in the gold-mining industry, and the subcutaneous method has practical advantages over the intravenous where prophylactic inoculation is to be carried out in man on the large scale which is necessitated by our conditions on the Rand. I proceeded therefore to investigate the subcutaneous method as applied to man.

In the first instance three East African Natives were each subcutaneously inoculated, on three occasions at seven days intervals, with the following doses: 1st inoculation, 2,000 millions; 2nd inoculation, 4,000 millions; 3rd inoculation, 4,000 millions. The sera of these Natives were tested during the course of inoculation and also seven days subsequent to the third inoculation and all failed to show, at any time and even with three volumes of serum, the presence of agglutinins. There was some irregular increase of opsonins, but this did not approach the standard that I regarded as satisfactory for my purpose.

Two East African Natives were next inoculated with the same number of doses and at the same time-intervals, but each dose now consisted of 12,000 million cocci. Tests performed in the usual manner afforded no better results than in the previous experiment.

The vaccine used on each occasion was of the same batch as had been employed throughout the intravenous experiment on man previously recorded; it had been prepared from blood-agar cultures. In view of this entire want of success I decided to repeat the experiments with a vaccine which, although composed of the same strains, had been prepared by a different method.

Pneumococci were grown in 4% goat-serum peptone-broth for about 18 hours; at the end of this period ·75% of glucose was added and the cultures were returned to the incubator for a further six hours. The presence of this percentage of glucose during the last six hours of incubation greatly increases the growth without bringing about that advanced degree of autolysis which frequently occurs when Pneumococci are grown in glucose broth for a continuous period of 24 hours or more.

The cocci were recovered by means of the centrifuge, and were emulsified in saline containing ·25% carbolic acid; this sufficed to render the vaccine sterile, no heat being applied for this purpose. The cocci were enumerated by Sir Almroth Wright's blood method.

The vaccine contained approximately 10,000 (ten thousand) million cocci per c.c., 2,000 millions of each of the Groups "A," "C," "D," "F," and "G."

Three freshly selected East African Natives were now inoculated as follows:—

TABLE XXVII.

Subcutaneous Inoculation of three East African Natives with a composite, serum-glucose, Vaccine. Dosage and Dates.

	May 9th (1st Inoculation).	May 16th (2nd Inoculation).	May 23rd (3rd Inoculation).
Native 864	10,000 millions (1 c.c.)	10,000 millions (1 c.c.)	10,000 millions (1 c.c.)
,, 794	20,000 millions (2 c.c.)	20,000 millions (2 c.c.)	20,000 millions (2 c.c.)
,, 769	30,000 millions (3 c.c.)	30,000 millions (3 c.c.)	30,000 millions (3 c.c.)

The site of the subcutaneous inoculation was a hand's breadth beneath the clavicle. The inoculum was administered, in the case of Native 794, by two, and in that of Native 769, by three, separate injections on each occasion.

The usual preliminary test of these Natives' sera, carried out prior to their first inoculation and using three volumes of the serum, disclosed entire absence of agglutinins throughout. The phagocytic counts were less than those of the European control.

Seven days after the first inoculation the presence of agglutinins and an increase in opsonins could be demonstrated in the serum of Native 769, who had received the largest dose; the evidences were, however, very slight.

Seven days after the second inoculation the agglutinins and opsonins had increased to a notable extent in the sera of Natives 794 and 769; these had received the larger doses. On this occasion the serum of Native 769 agglutinated and opsonised all strains, with the exception of "F," when only one volume of his serum was used. Number 864, who had received the smallest dose, had developed agglutinins to Groups "D" and "G" only.

In Table XXVIII. are presented the results obtained on testing the sera of these Natives, together with the serum of a European control (J.), seven days after the third subcutaneous inoculation with this vaccine (prepared from serum-glucose-broth cultures).

TABLE XXVIII.

(I Vol. Serum.)

Sera of Natives No. 864, 794, and 769 tested against Groups "A," "C," "D," "F," and "G," seven days after the Third Subcutaneous Inoculation with a composite, serum-glucose, Vaccine.

Strain.	Serum.	No. of Cocci in 25 Leucocytes.	Degree of Agglutination.
2480 ("A")	864	116	—
,,	794	Uncountable + + +	+ + +
,,	769	,, + + +	+ + +
,,	J (Control)	104	—
3472 ("C")	864	161	—
,,	794	157	—
,,	769	Uncountable — — +	+ + —
,,	J (Control)	42	—
300 ("D")	864	Uncountable	—
,,	794	,, + + +	+ +
,,	769	,, + + +	+ + +
,,	J (Control)	179	—
506 ("F")	864	Uncountable	—
,,	794	,,	—
,,	769	,,	—
,,	J (Control)	,,	—
6 ("G")	864	200	—
,,	794	Uncountable — —	+ +
,,	769	,, + + +	— — —
,,	J (Control)	103	—

Study of the foregoing Table shows that the largest doses had elicited the greatest antibody-response, and that the Native (864) who received the smallest dose failed to produce agglutinins to any of the five groups when one volume of serum was employed for the test.

This experiment was the first occasion on which I succeeded in producing agglutinins in man by subcutaneous inoculation. In order to confirm the results six other East African Natives were selected and, after the customary preliminary testing of their sera (which disclosed complete absence of agglutinins throughout), they were inoculated as follows:—

TABLE XXIX.

Subcutaneous Inoculation of six East African Natives with a composite, serum-glucose, Vaccine. Dosage and Dates.

	June 3rd (1st Inoculation).	June 9th (2nd Inoculation).	June 16th (3rd Inoculation).
Native 16174	20,000 millions (2 c.c.)	20,000 millions (2 c.c.)	30,000 millions (3 c.c.)
,, 16139	20,000 ,, (2 c.c.)	20,000 ,, (2 c.c.)	20,000 ,, (2 c.c.)
,, 16056	30,000 ,, (3 c.c.)	30,000 ,, (3 c.c.)	30,000 ,, (3 c.c.)
,, 16122	30,000 ,, (3 c.c.)	30,000 ,, (3 c.c.)	30,000 ,, (3 c.c.)
,, 16008	30,000 ,, (3 c.c.)	30,000 ,, (3 c.c.)	40,000 ,, (4 c.c.)
,, 15965	30,000 ,, (3 c.c.)	30,000 ,, (3 c.c.)	40,000 ,, (4 c.c.)

It will be noticed that the doses administered at the third inoculation were not serially consistent in all cases. In this experiment the site chosen for the inoculation was the same as in the preceding experiment, but the inoculum was now administered in every case by a single injection.

The results obtained confirmed the findings of the previous experiment, and the observations made seven days after the third, subcutaneous inoculation are recorded in Table XXX.; although somewhat lengthy this Table affords the reader the opportunity of detailed comparison with Table XXVIII.

TABLE XXX.

(1 Vol. Serum.)

Sera of Natives No. 16174, 16139, 16056, 16122, 16008, and 15965 tested against Groups "A," "C," "D," "F," and "G," seven days after the Third Subcutaneous Inoculation with a composite, serum-glucose, Vaccine.

Strain.	Serum.	No. of Cocci in 25 Leucocytes.	Degree of Agglutination.
2480 ("A")	16174	Uncountable	Trace
,,	16139	,,	—
,,	16056	,,	+
,,	16122	,,	+
,,	16008	,, + +	+ +
,,	15965	,, + + +	+ + +
,,	A.G.L. (Control)	127	—
3472 ("C")	16174	Uncountable	—
,,	16139	,, + + +	+ + +
,,	16056	,, + +	+ + +
,,	16122	,, +	+ +
,,	16008	,, + + +	+ + +
,,	15965	,, + +	+ +
,,	A.G.L. (Control)	22	—
300 ("D")	16174	Uncountable + +	+ + +
,,	16139	,, + + +	+ + +
,,	16056	,, + + +	+ + +
,,	16122	,, + +	+ +
,,	16008	,, + + +	+ + +
,,	15965	,, + + +	+ + +
,,	A.G.L. (Control)	154	—
506 ("F")	16174	Uncountable + +	+ +
,,	16139	,, + +	+ +
,,	16056	,, + +	+ + +
,,	16122	,, + + +	+ + +
,,	16008	,, + +	+ + +
,,	15965	,, + + +	+ + +
,,	A.G.L. (Control)	123	—
6 ("G")	16174	207	—
,,	16139	Uncountable +	+ +
,,	16056	,,	+ +
,,	16122	,,	+ +
,,	16008	165	Trace
,,	15965	Uncountable + +	+ + +
,,	A.G.L. (Control)	100	—

The results presented in the foregoing table correspond broadly with those recorded in Table XXVIII., they, however, reveal a good response to inoculation with Group "F" and a less satisfactory result, in some of the cases, where Groups "A" and "G" are concerned.

Selection and Preparation of Vaccine.

It will always be obligatory, where prophylactic inoculation against Lobar Pneumonia is in question, to ascertain which groups of the organism are prevalent in the population to be protected. This can only be compassed by a sustained investigation, or at least by investigations at comparatively short intervals. This necessity probably applies with greater force to the industrial population of the Rand than in America, Germany, and probably other countries.

As previously stated (under the section dealing with the Classification of Pneumococci) there are a greater number of groups in the Transvaal than in some other countries. Not only is this the case, but the prevalence of some of the less important groups peculiar to South Africa appears to change from year to year; without precise knowledge as to the groups which should be represented in our vaccine any attempt at extended prophylactic inoculation will be at a serious disadvantage.

For use on the Rand a prophylactic vaccine should, at the present time, include a due representation of Groups "A," "B," and "C." It will also be advantageous to make use of those strains from the selected groups which experiment has shown to possess the best antigenic qualities, and it is not improbable that the most satisfactory results will generally be achieved with recently isolated cultures.

If the intravenous method of inoculation is to be used I would recommend that the vaccine should be prepared from 24-hour growths of Pneumococci on blood-agar. For vaccine for subcutaneous administration the Pneumococci should be obtained from a serum-glucose-broth culture, in the manner described in the section "Subcutaneous Inoculation of Pneumococcal Vaccine in Man."

When many tens of thousands of these relatively large doses of pneumococcal vaccine are required, practical difficulties arise in its rapid manufacture. I have attempted to circumvent these inconveniences by experiments in a number of directions, but hitherto with but little success; recourse has been had to—(1) filtering broth cultures rapidly through Berkefeld filters, (2) separation of the cocci from broth cultures by electrolytic methods, (3) precipitation of the cocci by means of alum, and (4) using for the vaccine the heavy deposit of Pneumococci which occurs in glucose-broth cultures after 40 hours' incubation.

With methods (1) and (2) I obtained no success; with (3) the cocci may be rapidly recovered, but at present I have not determined whether a vaccine prepared by this method will elicit the necessary antibody-formation. With (4) it is possible rapidly to prepare very large quantities of vaccine, but unfortunately the material has proved, on

experiment, to be quite inert as an immunising agent; the cocci obtained by this method have undergone advanced autolytic changes which apparently completely destroy their antigenic properties. At the present time the only practical method of preparing the vaccine in large quantities for subcutaneous administration is by recovering the cocci from glucose-serum-broth cultures by means of large centrifuges.*

It is not out of place here to remark that the rabbit and the man do not respond similarly to injections of the same pneumococcal vaccine. The subcutaneous administration, *to a man*, at appropriate intervals, of three 30,000 million doses of a composite (five-group) vaccine—prepared from serum-glucose-broth cultures—will result, as already shown (Table XXVII. *et seq.*) in satisfactory antibody-production; smaller doses of the same vaccine will be equally successful if administered intravenously. A *rabbit* can, however, be inoculated with doses of exactly the same size of the same vaccine, either subcutaneously or intravenously, and yet no appearance of agglutinins or increase of opsonins will become apparent. An entirely satisfactory immunity-response can nevertheless be elicited in the rabbit by the intravenous administration of only 1,000 million cocci (Table XII. *et seq.*) of a composite (five-group) vaccine which has been prepared from 24-hour blood-agar cultures.

These facts indicate the need for the further investigation of pneumococcal antigens, and suggest the possibility that improved methods will yield better results than are recorded in this study.

Dose and Method of Administration Necessary to Produce Agglutinins and Opsonins for the Pneumococcus in the Serum of Man.

It makes but little difference to the resulting antibody-formation in the serum of man whether the vaccine be administered intravenously or subcutaneously, provided that suitable dosage be employed for the method chosen; a dose that will give satisfactory results if injected intravenously may be without demonstrable effect if given subcutaneously, but not *vice versa*.

It appears to be of no importance whether a given dose be administered in a single or in multiple injections at the same time. It is, however, essential to a satisfactory result that more than one dose be given; it is true that it is possible to demonstrate the presence of agglutinins in the serum of man after one large intravenous dose, or even after a single very large subcutaneous dose, and the agglutinin content of the serum increases after two substantial subcutaneous injections, yet the most constant and satisfactory results follow the third dose.

* Since writing the above I have discovered that the Pneumococci growing in a broth culture are, *under certain easily attainable conditions*, retained in the revolving bowl of a particular type of milk-separator, and I have adopted this method for the preparation of this vaccine. My chief difficulty now lies in the comparative scantiness of the pneumococcal growth obtainable in fluid media.

The doses should be spaced at seven-day intervals. Although results equally good are obtained where the intervals are of five days I think it advisable to allow, in man, the extra two days to ensure the complete disappearance of any feeling of malaise or of any local reaction which may occasionally supervene upon an inoculation.

Having in mind the practical advantages possessed by the subcutaneous method as compared with the intravenous—not only in the saving of time but also in its freedom from certain theoretical dangers—I recommend the following procedure:—

> Three doses, each containing 6,000 (six thousand) millions of each group of Pneumococcus against which immunity is sought to be established, should be inoculated subcutaneously in the infraclavicular region, at intervals of seven days.

Toxicity of Pneumococcal Vaccine to Animals.

Killed pneumococcal vaccines have proved but slightly toxic to experimental animals; all the inoculated rabbits, even where three intravenous doses each of 20,000 million cocci were employed, increased in weight during the period of immunisation. The constancy of this feature was doubtless in part due to the fact that the animals about to be inoculated were transferred from a large outside run to small cages inside the building, thus curtailing their activity.

During my investigations I also inoculated three guinea-pigs as follows:—

No. 1.—Three subcutaneous inoculations, each of 60,000 million cocci, at seven days' interval.

No. 2.—Three subcutaneous inoculations, each of 40,000 million cocci, at seven days' interval.

No. 3.—Three subcutaneous inoculations, each of 30,000 million cocci, at seven days' interval.

These large doses did not appear to be in any way detrimental to the health of any of these three animals. Two months later one of them had a litter of quite healthy young.

General and Local Reaction in Man following Pneumococcal Inoculations.

It is necessary to point out that the temperature of East African Natives frequently varies, for reasons I think but little understood. If the temperature of a batch of such Natives, apparently in normal health, be taken twice daily over a period of a month a number of them will show frequent slight elevations.

The Natives inoculated intravenously showed no local and very slight general reaction, and none was prevented from continuing his usual work; it should be remarked, however, that they were employed in surface work, which is less arduous than many forms of underground employment. Similarly, in my own case, the general reaction was mild after the first, and absent after the second and third doses, and caused no interference with my usual occupations.

The Natives who had been inoculated subcutaneously usually showed a moderate local reaction, but none complained of subjective symptoms. The Native, however, is not usually easily disturbed by anything short of a serious illness, and will often protest that there is nothing amiss with him, even when his temperature registers 103°F. The general reaction may be said to be usually less pronounced than that following prophylactic inoculation for Enteric Fever.

It is not altogether a digression to record here that I have given doses of 2,500 and 5,000 millions of cocci, intravenously and thrice repeated, to Native patients in the acute stages of Lobar Pneumonia. At present I have a series of only seven such cases, all of which ended in recovery, but I do not in the least desire to draw any conclusion from this small number as to the efficiency of vaccine in the treatment of Lobar Pneumonia. The two points of special interest about these seven patients were that they elaborated agglutinins and opsonins for the five strains inoculated, and that no serious negative phase was developed. The latter observation may perhaps encourage the trial of pneumococcal vaccine, *administered in effective doses*, in the treatment of Lobar Pneumonia.

Duration of Results of Inoculation.

I am not in a position to estimate confidently the duration of the blood-changes produced by the method of inoculation advocated in this paper.

It appears probable that some degree of immunity remains after the disappearance of agglutinins, and after the opsonins have returned to the normal amount; this is said to hold in anti-enteric inoculation. The slight degree of immunity which was conferred upon 28,000 Natives[19] by a single and altogether inadequate dose of composite vaccine could be recognised as lasting for a period of five months; in this "Inoculation Experiment" the immunity, such as it was, must have been associated with alterations in the serum altogether too small to be detected by the most refined laboratory investigation.

Six months have now elapsed since my own inocualtion and my serum still contains agglutinins and increased amounts of opsonins for various groups of Pneumococcus. My serum is, however, not nearly so rich in these antibodies as was the case four months after the inoculations, and I anticipate that at the end of seven months it will

be impossible to demonstrate them.* The inoculations which I received were, however, inadequate as regards dosage; all those individuals who were subsequently given larger doses developed in their sera greater amounts of antibodies than in my own case. Insufficient time has elapsed to show whether the characteristics in the sera of these other individuals will outlast those in my own; it is probable that they will. For the same reason I cannot yet form an opinion as to whether the antibodies elicited by the large subcutaneous inoculations are more persistent than those developed in response to a much smaller intravenous inoculation.

From a general consideration of these facts I conclude that some degree of immunity will be apparent for at least a year after adequate prophylactic inoculation.

Discussion.

In the section dealing with the antibodies which serve to gauge immunity response I have recorded the opinions of a number of workers who attribute a very important rôle to phagocytosis and agglutination in the mechanism of recovery from pneumococcal infections; in my own experience quite 90% of pneumonic patients have in their post-critical sera the antibodies necessary to demonstrate these phenomena in connection with the Pneumococci responsible for their infection. If it be admitted that Pneumococci are destroyed in the pneumonic patient largely through the agency of these two antibodies, it seems a justifiable assumption that a man whose serum displays these same characteristics as a result of prophylactic inoculation will be able to resist infection by those Pneumococci which are immunilogically related to the specific group-antibodies in his serum.

It is often pointed out that a patient who has recovered from an attack of Lobar Pneumonia is not less, but more, susceptible to a second attack; there are too many such instances recorded to leave this point in doubt[18], and it is therefore argued that there is not much hope for the success of any prophylactic immunisation of man.

Another adverse speculation occasionally advanced is that Lobar Pneumonia is a localised disease, and that the successful immunisation of animals against Pneumococcal Septicaemia does not justify the assumption that a similar immunisation in man will enable him to resist Lobar Pneumonia.

To deal with the former difficulty first. There are two lines of argument that have a bearing upon this aspect; they are distinct from

* More than two months have passed since this expression of opinion was committed to writing; I find, at the present time (October 28th)—eight calendar months since my last inoculation—that the agglutinating power of my serum is not demonstrably less than was the case at the end of six months. It seems possible that a stage has been reached at which the agglutinin-content of my serum will remain constant for a further considerable period.

each other, but neither of them need prejudice our conception of the probable efficacy of the immunity established by prophylactic inoculation.

(1) I have stated in a previous communication[4]: "The opsonins and agglutinins seem to disappear from the freshly drawn serum at the end of a few weeks." This statement referred to the opsonins and agglutinins naturally elicited in the serum of a pneumonic patient about the time of his crisis. With a wider experience I am now aware that opsonins and agglutinins elaborated in the course of an attack of Lobar Pneumonia occasionally persist in the serum of the patient for several months after recovery. I have under observation at the present time a Native whose serum opsonises and agglutinates all strains belonging to the group organism "C," a strain belonging to this group having given rise to his attack of Lobar Pneumonia more than four months ago. Notwithstanding the occurrence of these exceptional cases, it may be stated generally that the presence of these two antibodies in the sera of pneumonic patients can be demonstrated, in the great majority of cases, for a very brief period only. More recently, Chickering[16] investigated the serum in forty cases of Pneumonia. In 30 of these cases agglutinins were present in the serum at about the time of the crisis; in one case agglutinins persisted for 84 days; in four others for one day only; whilst in the remaining 25 the longest duration of agglutinating power was twenty days and the average duration was but seven days. The short-lived duration of these "infection" antibodies stands in very marked contrast to the persistent duration of the antibodies elaborated in the serum of the rabbit and man in response to *prophylactic inoculation with pneumococcal vaccine*; and I have observed that they persist for a longer period in man than in the rabbit. My own case has already been referred to, and I have noted the presence of agglutinins in my serum eight months after inoculation, together with the prospect of their persisting still longer. Similar investigations carried out with the serum of both the rabbit and man indicate that this persistence of agglutinins is the rule rather than the exception, more particularly in the case of man. It will now be apparent that the antibodies established by an attack of Lobar Pneumonia differ very essentially as regards their persistence from those induced by inoculation. May it not then be a reasonable assumption that second attacks of Lobar Pneumonia supervene in those individuals from whose serum the antibodies have rapidly disappeared?

It is interesting to speculate upon the cause of this somewhat sudden disappearance of these "infection" agglutinins in many cases of Lobar Pneumonia. I suggest that the phenomenon may be due to a using up of the opsonins and agglutinins by the vast number of Pneumococci which must be present in a disease which is frequently associated with such a massive lesion. It is to be remembered that in cases of Lobar Pneumonia the serum of the patient has in many instances reached its

highest agglutinating titre shortly after the crisis, and that this event may occur only three or four days after the initial rigor; in such circumstances the tissues of the patient have made their maximal response, and if all the antibodies generated are absorbed by the cocci still present in the tissues immunity can be but brief. The tissues may, for the time, be unable to elaborate further antibodies to the residual Pneumococci, or the protein of these residual organisms may have undergone some alteration depriving it of its antigenic qualities.

(2) The second line of argument respecting second attacks of Lobar Pneumonia is concerned with the specific immunity associated with the various groups of Pneumococci; and in this connection it is of interest to note that an instance of a second attack of Lobar Pneumonia has been bacteriologically investigated by Mathers[17], he states—"In this case there were two different pneumococcus infections caused by organisms with different biologic reactions, the first infection was caused by an organism with the biologic characteristics of Group 1.; the terminal infection was caused by a member of the heterologous group." In discussing the case he further says—"Is such an occurrence a new infection by a different organism, or a variation of the organism causing the first infection? Or may it be a latent process activated by the debilitating effects of the first attack?" He inclines to the second explanation and considers that this was, in other words, an instance of mutation. Although I think that the arguments against this explanation outweigh those in favour of it, yet the facts stated clearly show, that in this case a second attack of Lobar Pneumonia was associated with a Pneumococcus differing entirely in its biologic characteristics from the one responsible for the first attack. This incidentally illustrates how the scope of investigation has been extended by the classification of Pneumococci; the application of the principal to the problem of second attacks, which has now been recorded for the first time, casts doubt upon the hypothesis, hitherto accepted, that they necessarily arise through a breaking down of the immunity established by the first attack.

An experience of my own, whilst not affording quite such positive evidence, was as follows:—I obtained by lung-puncture, from a Native pneumonic patient, a culture of Pneumococcus, and identified it by means of stock sera as belonging to my Group "C." The post-critical serum of this patient subsequently agglutinated not only this strain but also, though to a less degree, members of Group "A." A post-critical serum capable of agglutinating Pneumococci of a group other than the one causing the infection had never previously come within my experience, nor has it since, with the exception of those seven cases of Lobar Pneumonia previously referred to and which received large intravenous inoculations of a polyvalent group-vaccine. Upon enquiry, this Native stated that he had an attack of some chest disease, whilst resting at his kraal, similar to that he was now suffering from, and that as soon as he recovered he came up to the mines where he had been resident one month. It seems to me highly probable that this

Native, whilst at his kraal, had suffered from Pneumonia due to Pneumococcus Group "A," and that about two months later he had a second attack due to Pneumococcus Group "C." We need not then be discouraged by the hypothesis which seeks to explain these second attacks by a failure of the immunity established by the first; their occurrence may be capable of a more probable explanation, an explanation based upon experimental investigation and more in keeping with ascertained facts concerning immunity in general.

Now as regards the speculation that an immunity similar to that which protects a rabbit from Pneumococcal Septicaemia will not necessarily protect a man from Lobar Pneumonia—It is to be remarked that in the majority, if not in all, cases of Lobar Pneumonia in man there co-exists at some stage of the disease a blood-infection, and that in the majority of cases man's natural immunity overcomes this septicaemia. In the rabbit, however, it is extremely rare for recovery to ensue after invasion of the blood-stream by Pneumococci belonging to the disease-producing groups in man. Man has therefore—and this is generally conceded—a greater natural resistance to the Pneumococcus than is possessed by the rabbit, and it is this fact which is probably responsible for the phenomenon of pulmonary localisation of the disease in man. We know that a contact may harbour in his pharynx the disease-producing types of Pneumococcus, and that these may disappear without having given rise to Pneumonia.[20] Similarly, the Pneumococci which have caused Pneumonia persist for a time in the pharynx of a patient who has recovered, disappearing at varying periods after the crisis[20] owing probably to the presence of immune substances in the blood of such patient. But little is definitely known as to the route by which the Pneumococcus gains entrance into the lung, yet it is hardly credible that natural infection in man consists in his being suddenly submitted to an overwhelming dose of Pneumococci, a dose comparable in magnitude with that which is purposely inoculated into a rabbit in the course of a protection experiment. It seems more reasonable to assume that in man a small number of Pneumococci gain entrance to the circulation, and only give rise to Pneumonia if they survive a stubborn struggle for existence; the issue of the conflict is in doubt right up to the end, and frequently culminates in the destruction of the Pneumococcus before it has brought about a gross pulmonary lesion and thus literally consolidated its position in the body.

From these considerations it appears to me probable that the inoculated rabbit, in overcoming the otherwise inevitably lethal dose which has been administered for the purpose of a protection test, has a more difficult task than the inoculated man who receives a natural infection; the latter is called upon to destroy only those few Pneumococci which gain access to his circulation by natural means, and he very frequently succeeds even when he has not been inoculated. In the man the production of Lobar Pneumonia by the invaders is not an inevitable event, even if he be uninoculated, but only a possibility.

The mere question of the localisation of the disease in the human lung cannot therefore, I think, be a serious objection; if the prophylactic inoculation acts successfully the battle will be decided long before hepatisation can ensue and render the cocci inaccessible to the action of the antibodies of the blood; if the prophylactic inoculation fails it will not be on account of the pulmonary lesion having been established.

It has been admitted that the incidence of Lobar Pneumonia amongst the very large groups of inoculated Native labourers on the Rand has shown a diminution as compared with the control groups[18]. Calculations made from the results of the last experiment* on the Rand[19] set down the diminution at about 25%, over a period of five months, in favour of the inoculated. The procedure adopted in this experiment consisted in one subcutaneous injection of 2,000 (two thousand) million cocci comprising five groups. Such a dose does not give rise to either agglutinins or opsonins demonstrable by the technique employed by me; the degree of protection conferred by this procedure appears nevertheless to have been statistically quite appreciable.

With the method of inoculation now devised—one which ensures the formation of both agglutinins and opsonins in substantial and recognisable amounts in the sera of the inoculated—I anticipate results far better than those obtained in previous "Inoculation Experiments."

In the system hitherto employed on the Rand for assaying the results of prophylactic inoculation against the Pneumococcus a certain advantage conferred upon the uninoculated has, I think, been overlooked. If Pneumonia is spread, as I believe it to be, either directly from case to case or through the agency of carriers it follows that the inoculation of half the inhabitants of a Native compound may interrupt the chain, not only of actual pneumonic patients, but also of carriers; if the inoculation achieves this it is obvious that the uninoculated half of the population will reap an advantage which is not allowed for in the calculations.

If the problem to be studied had been the immunisation of rabbits against pneumococcal infection one might say that it has been satisfactorily solved and the results put to a crucial test. I have shown that suitable inoculation will give rise in man to those antibodies which appear to be mainly responsible for the immunity which can be established in the rabbit. Dictates of humanity preclude the application of a crucial test to men who have been inoculated; it may nevertheless be reasonably anticipated that the resistance of an inoculated man to a natural infection by the Pneumococcus will be as satisfactory as that of the inoculated rabbit to an artificial infection—owing to the presence in his serum of the same demonstrable antibodies.

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* In this experiment approximately 28,000 Natives were inoculated, and a similar number were used as controls.

It is obviously of first importance that a suitable experiment should be organised to put this anticipation to the test. This can only be done by inoculating a group of individuals "at risk" and recording the subsequent incidence of Pneumonia amongst them. In such an experiment it will be necessary to ascertain whether cases of Pneumonia which may arise amongst the inoculated are or are not due to organisms belonging to the groups comprising the vaccine; it would be manifestly illogical to expect protection against any group of Pneumococcus of the Lobar-Pneumonia-producing type which was not represented in the vaccine used.

In concluding this record it is but right that I should specially mention the names of some of those to whom I am indebted for assistance. The Director of the Institute, Dr. W. Watkins-Pitchford, has not only placed all possible facilities at my disposal but has followed the details of the work with close personal interest; he has made many suggestions and has extended a very helpful encouragement throughout.

Dr. G. A. Turner has assisted by securing a number of Natives for inoculation and observation, and many Mine Medical Officers have extended valuable co-operation by supplying material and enabling me to obtain cultures.

Miss Alice G. Lambert, my Laboratory Assistant, has helped me throughout the whole investigation. The reliability and accuracy of her work, and her loyal devotion to her duties, are deserving of high commendation.

Mr. A. W. Hall, superintendent of the Bantjes Gold Mine Native Hospital, has given me valued assistance in connection with the inoculation of Natives and the collection of specimens.

Conclusions.

1. More than sixty *per cent.* of the cases of Lobar Pneumonia amongst Natives on the Rand are associated with but two, though not always the same two, groups of Pneumococcus. Group "A" is constantly the most prevalent, the second position is occupied sometimes by Group "B," at others by Group "C."

2. There are at least four groups of Pneumococcus on the Rand which are additional to those recognised by Dochez and Gillespie in America.

3. Immunilogical reactions associated with these various groups are specific for each group.

4. Normal rabbit or normal human serum is incapable of agglutinating virulent Pneumococci derived from cases of Lobar Pneumonia, even under the most favourable test conditions.

5. Agglutinins and opsonins are readily produced and easily demonstrated in the blood of rabbits and men subsequent to inoculation with suitable doses of killed Pneumococci.

6. The method employed by Sir Almroth Wright in his opsonic technique, provided that the volumes of serum are varied, is sufficiently practical and accurate for following the progress of pneumococcal immunisation.

7. Very much smaller doses of killed Pneumococci will elicit the formation of agglutinins and opsonins, both in rabbit and man, when the inoculum is injected into a vein than when subcutaneous inoculation is employed.

8. Killed pneumococcal vaccines have very slight toxicity; subcutaneous inoculation of forty thousand million cocci usually causes little reaction in either the guinea-pig, rabbit, or man. Intravenous inoculation of twenty thousand million cocci in the rabbit, and forty thousand million cocci in man, have given rise to but very slight reactions.

9. Antibody-formation is greatly increased after a second inoculation and rises to a still higher degree after a third inoculation.

10. Rabbits inoculated three times with killed pneumococcal vaccine, in suitable doses, and having agglutinins and opsonins demonstrable in their sera, are resistant to at least eight times the lethal dose of living Pneumococcus (homologous group) when inoculated subcutaneously.

11. Agglutinins and opsonins are intimately concerned in the mechanism of resistance against pneumococcal infections.

12. Pneumococcal vaccines prepared from glucose-serum-broth cultures are less satisfactory antigens in the rabbit than in man.

13. Pneumococci that have sedimented in a 40-hour old glucose-serum-broth culture are devoid of antigenic properties both for rabbit and man.

14. For prophylactic purposes in man the subcutaneous method should be adopted. Three inoculations, at seven days' interval, should be employed; each dose should consist of 6,000 (six thousand) million cocci of each group against which immunity is desired.

15. Three intravenous inoculations of pneumococcal vaccine have been given in each of seven cases of Lobar Pneumonia during the acute stage. The treatment has not given rise to any clinically recognisable negative phase; all the patients recovered and were found to have elaborated antibodies to the strains which comprised the vaccine.

JOHANNESBURG,
October 1, 1916.

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BACTERIOLOGICAL NOTES & OBSERVATIONS.

By A. R. Friel and F. S. Lister.

These investigations were in progress during 1915, when Dr. Friel, answering the call of patriotism, abandoned the work before our observations could be amplified or conclusive results arrived at.

An increased amount of interest is now being shown in the changes which can be brought about in micro-organisms by exposing them to the influence of immune serum. Laura M. Stryker[1] has published certain experimental results which confirm the observations previously published by one of us (A.R.F.)[2], and it is thought that the following brief record, though of admittedly incompleted studies, may prove of interest to those engaged on similar lines of investigation.

I.

A Note on the Effect produced on the Opsonic Property of immune and normal Serum by the addition of Serum containing the Products of autolysed Bacteria.

In the carrying out of this work it was necessary to prepare immune sera, and to determine their efficiency by means of agglutination and opsonic tests. These tests were made by means of phagocytic mixtures, and as they were required to afford only approximate indications, their exact quantitative details were not elaborated.

With regard to such tests, it is to be remarked that they deal with certain of the properties of serum which are developed in response to the inoculation of bacteria in their entirety rather than with any property which might arise from the inoculation of toxin or other soluble product of bacterial action or lysis.

It is conceivable that when an animal receives an injection of bacteria the first event is an anchoring and gradual dissolution of the bacterial bodies, and that the later efforts of the tissues are directed to elaborating substances which will neutralise the products of the dissolved bacteria. Agglutination and opsonic tests furnish an indication of the ability of the organism to affect bacteria in their entirety, but do not necessarily demonstrate its power to deal with the bacterial products in solution. In seeking for some index of this latter power, use was made of the serum of animals which had received several injections of a strain of B. coli communis, because the serum of these animals produced lysis of the organism.

A Wright's capsule was filled with the immune rabbit's blood, obtained by puncturing a vessel in the ear with a needle. When the blood had clotted the serum was pipetted off, and a considerable amount of a young agar culture of the antigen strain of B. coli communis was suspended in it. The mixture was allowed to remain in the incubator at 37° for four or five hours; the tube was then centrifugalised and the supernatant serum separated from the bacterial deposit. This supernatant serum—which, for shortness, we shall now call "L.B.S."

(lysed bacterial serum)—contained the soluble substances which resulted from the lysis of the bacterial bodies.

We now endeavoured to ascertain the effect, if any, of the use of this L.B.S. in phagocytic mixtures containing respectively (*a*) normal serum, (*b*) immune serum, and (*c*) no serum other than the L.B.S.

When carmine particles were used to indicate the relative amount of phagocytosis in the various phagocytic mixtures it was found that both in "a" and in "b" ("a" = L.B.S. + normal serum + corpuscles + carmine, and "b" = L.B.S. + immune serum—*i.e.*, rabbit "colon" serum—+ corpuscles + carmine) practically no phagocytosis occurred; whereas in "c" (= L.B.S. + saline + corpuscles + carmine) a fair amount of phagocytosis was observed.

The suspension of carmine which we employed proved unsatisfactory; the particles varied greatly both in size and outline, and the material, moreover, was found to be capable of being phagocyted by corpuscles in a medium of normal saline alone. Furthermore, it was often impossible to decide whether a particle under observation was single or was an aggregation of several smaller particles. For these reasons it became obvious that no reliance could be placed upon the results of phagocytic enumerations when carmine was employed; the broad difference noted above had been, however, definitely established by its use.

In consequence of these shortcomings with suspensions of carmine, we decided to employ suspensions of the Micrococcus pyogenes aureus. Many tests were made on these lines, and it was found (1) that there was no evidence of morphological change in the white blood cells* as a result of the action of L.B.S., (2) that the phagocytic count was *reduced* by the addition of L.B.S., and (3) that this reduction was very marked in the presence of "immune serum."

TABLE I.

Opsonic effect of normal serum, immune serum, and L.B.S. respectively.

	Constituents of Phagocytic Mixture. (Incubated together for 1 hour at 37° C.)	Added and incubated for ½ hour.	Cocci in 25 Leucocytes.
a.	1 vol. saline + 2 vols. normal serum (A.R.F.) + corps.	1 vol. emulsion of M.p.a.	75
b.	1 vol. saline + 2 vols. immune serum (rabbit) + corps	..	35
c.	2 vols. saline + 1 vol. L.B.S. + corps	..	41

* The corpuscles used throughout all these experiments were those of human (European) blood.

When the saline in "a" and "b" was replaced with similar volumes of L.B.S. the counts were, respectively, 51 and 19; that is to say, phagocytosis was markedly reduced, more particularly in the presence of immune serum. This is shown in the following table:—

TABLE II.

Opsonic effect when L.B.S. is substituted for normal saline.

	Constituents of Phagocytic Mixture. (Incubated together for 1 hour at 37° C.)	Added and incubated for ¼ hour.	Cocci in 25 Leucocytes.
a.	1 vol. L.B.S. + 2 vols. normal serum (A.R.F.) + corps	1 vol. emulsion of M.p.a.	51
b.	1 vol. L.B S. + 2 vols. immune serum (rabbit) + corps	,,	19

Despite the result recorded in Table I., it is to be remarked that the capacity of the L.B.S., by itself, to opsonise the Micrococcus varied considerably: sometimes it was fairly marked and sometimes almost absent.

The immune serum employed was, as already stated, that of a rabbit immunised with a strain of B. coli communis.

These experiments were subsequently repeated on many occasions, and although the capacity of L.B.S. to inhibit phagocytosis was not always of the same degree, it was nevertheless quite apparent on every occasion.

The following records of actual experiments exemplify the findings usually obtained in these subsequent observations. The opsonic power of the immune B. coli and normal rabbits' sera towards Micrococcus pyogenes aureus was again first ascertained, as follows:—

TABLE III.

Constituents of Phagocytic Mixture. (Incubated ¼ hour at 37° C.)	Cocci in 25 Leucocytes.
1 vol. Immune Rabbit's serum + 1 vol. corps. + 1 vol. cocci	65
1 vol. Normal ,, ,, + 1 vol. ,, + 1 vol. ,,	82

In the experiment recorded in Table IV. varying volumes of immune (B. coli) or of normal rabbit's serum were severally mixed with 1 vol. of L.B.S. and were incubated for one hour at 37°C.; phagocytic mixtures were next prepared, the constituents of which, in each case, were—1 volume of these incubated L.B.S. + serum mixtures, 1 volume of corpuscles, and 1 volume of cocci.

TABLE IV.

	Incubated 1 hr. at 37° C.	Constituents of Phagocytic Mixture (Incubated quarter of an hour at 37° C.).			Cocci in 25 Leucocytes.
1	1 vol. L.B.S. + 1 vol. I.S.	1 vol. preceding mixture.	+ 1 vol. corps.	+ 1 vol. cocci	27
2	1 vol. L.B.S. + 1 vol. N.S.	1 vol. preceding mixture.	+ 1 vol. ,,	+ 1 vol. ,,	46
3	1 vol. L.B.S. + 2 vols. I.S.	1 vol. preceding mixture.	+ 1 vol. corps.	+ 1 vol. cocci	30
4	1 vol. L.B.S. + 2 vols. N.S.	1 vol. preceding mixture.	+ 1 vol. ,,	+ 1 vol. ,,	58
5	1 vol. L.B.S. + 3 vols. I.S.	1 vol. preceding mixture.	+ 1 vol. corps.	+ 1 vol. cocci	13
6	1 vol. L.B.S. + 3 vols. N.S.	1 vol. preceding mixture.	+ 1 vol. ,,	+ 1 vol. ,,	52
7	1 vol. L.B.S. + 6 vols. I.S.	1 vol. preceding mixture.	+ 1 vol. corps.	+ 1 vol. cocci	10
8	1 vol. L.B.S. + 6 vols. N.S.	1 vol. preceding mixture.	+ 1 vol. ,,	+ 1 vol. ,,	39

(L.B.S. = lysed bacterial serum. I.S. = immune rabbit's serum. N.S. = normal rabbit's serum. Corps. = corpuscles derived from F.S.L. Cocci = Micrococcus pyogenes Aureus.)

In the experiment recorded in Table V. a control was arranged, viz., the addition of 1 volume of normal saline to varying volumes of sera; the conditions as regards incubation and volumes used in the phagocytic mixtures were the same as in the preceding experiment.

TABLE V.

	Incubated 1 hr. at 37° C.	Constituents of Phagocytic Mixture (Incubated quarter of an hour at 37° C.).			Cocci in 25 Leucocytes.
1	1 vol. L.B.S. + 1 vol. I.S.	1 vol. preceding mixture.	+ 1 vol. corps.	+ 1 vol. cocci	6
2	1 vol. L.B.S. + 1 vol. N.S.	1 vol. preceding mixture.	+ 1 vol. ,,	+ 1 vol. ,,	10
3	1 vol. saline + 1 vol. I.S.	1 vol. preceding mixture.	+ 1 vol. ,,	+ 1 vol. ,,	41
4	1 vol. saline + 1 vol. N.S.	1 vol. preceding mixture.	+ 1 vol. ,,	+ 1 vol. ,,	61
5	1 vol. L.B.S. + 3 vols. I.S.	1 vol. preceding mixture.	+ 1 vol. corps.	+ 1 vol. cocci	2
6	1 vol. L.B.S. + 3 vols. N.S.	1 vol. preceding mixture.	+ 1 vol. ,,	+ 1 vol. ,,	33
7	1 vol. saline + 3 vols. I.S.	1 vol. preceding mixture.	+ 1 vol. ,,	+ 1 vol. ,,	70*
8	1 vol. saline + 3 vols. N.S.	1 vol. preceding mixture.	+ 1 vol. ,,	+ 1 vol. ,,	68
9	1 vol. L.B.S. + 6 vols. I.S.	1 vol. preceding mixture.	+ 1 vol. corps.	+ 1 vol. cocci	5
10	1 vol. L.B.S. + 6 vols. N.S.	1 vol. preceding mixture.	+ 1 vol. ,,	+ 1 vol. ,,	28
11	1 vol. saline + 6 vols. I.S.	1 vol. preceding mixture.	+ 1 vol. ,,	+ 1 vol. ,,	42
12	1 vol. saline + 6 vols. N.S.	1 vol. preceding mixture.	+ 1 vol. ,,	+ 1 vol. ,,	52

* Marked agglutination of the cocci was also present.

An experiment on similar lines—but in which we took certain precautions in order to eliminate as far as possible any unconscious bias—afforded the results detailed in Table VI. The method adopted in this experiment was to have each slide *lettered* by a third person, who kept a key to the corresponding experimental number of the slide, and this latter was not disclosed until the counting had been completed. Further, in this instance, 50 leucocytes were counted in every slide by each of us, and the mean of the two counts was taken as the result to be recorded.

Five mixtures were prepared, each of which contained one volume of L.B.S. and three volumes of serum—the three volumes of serum being derived from B. coli-immune rabbits Nos. "0," "10," "12," and "14," and from a normal rabbit, respectively. These five mixtures were then incubated at 37°C. for one hour. One volume of each of these incubated mixtures, together with one volume of corpuscles and one volume of cocci, constituted one of a set of five phagocytic mixtures which demonstrated the influence of the L.B.S.; whilst one volume of the serum of each corresponding rabbit, together with one volume of corpuscles and one volume of cocci constituted one of a set of five other phagocytic mixtures which showed the ordinary opsonising power of the same sera. An estimation of the opsonic power of L.B.S. alone towards Micrococcus pyogenes aureus was first made, as follows:—

Constituents of Phagocytic Mixture. (Incubated ½ hr. at 37° C.)	Cocci in 50 Leucocytes.
1 vol. L.B.S. + 1 vol. corps. + 1 vol. cocci	64

The results of the experiment carried out on the lines described were as follows:—

TABLE VI.

	Constituents of Phagocytic Mixture. (Incubated ¼ hr. at 37° C.)			Cocci in 50 Leucocytes.
1	1 vol. (L.B.S. + serum Rabbit "O")	+ 1 vol. corps	+ 1 vol. cocci	130
2	1 vol. serum alone Rabbit "O"	+ 1 vol. ,,	+ 1 vol. ,,	300
3	1 vol. (L.B.S. + serum Rabbit "10")	+ 1 vol. corps	+ 1 vol. cocci	113
4	1 vol. serum alone Rabbit "10"	+ 1 vol. ,,	+ 1 vol. ,,	256
5	1 vol. (L.B.S. + serum Rabbit "12")	+ 1 vol. corps	+ 1 vol. cocci	200
6	1 vol. serum alone Rabbit "12"	+ 1 vol. ,,	+ 1 vol. ,,	324
7	1 vol. (L.B.S. + serum Rabbit "14")	+ 1 vol. corps	+ 1 vol. cocci	123
8	1 vol. serum alone Rabbit "14"	+ 1 vol. ,,	+ 1 vol. ,,	325
9	1 vol. (L.B.S. + serum Rabbit "Normal")	+ 1 vol. corps	+ 1 vol. cocci	201
10	1 vol. serum alone Rabbit "Normal"	+ 1 vol. ,,	+ 1 vol. ,,	198

It is to be noted that the L.B.S. in the above experiments possessed a certain degree of opsonizing power on the Micrococcus, and that its inhibitory action was not so strongly marked as in some of the preceding experiments; the effect, however, was quite considerable.

We now sought to ascertain whether this inhibitory action of L.B.S. could be utilised either as a measure of the amount of immunity conferred upon our inoculated animals, or as a diagnostic aid in infected human beings.

In the former case we expected to find that the serum of an animal in process of immunisation would (*a*) provide L.B.S. of a degree of activity proportioned to its immunity response, and (*b*) that its opsonic power towards the M. pyogenes aureus, after admixture and incubation with equal quantities of a standard L.B.S., would vary proportionately to the immunity response. In the latter case,

taking Enteric Fever as an example, we expected to find that the serum of an infected individual, upon addition of Enteric L.B.S., would show a marked difference in its phagocytic power towards Micrococcus pyogenes aureus as compared with that of a normal serum under similar conditions.

In a number of experiments carried out to these ends our results, both with respect to the gauging of the degree of immunity conferred upon our animals and the use of the phenomena as a diagnostic procedure, were too discordant to warrant the drawing of any definite conclusions; we are of the opinion, however, that further study of this phenomenon may lead to its better understanding and perhaps to some practical application.

At the present time we consider that the phenomenon of the inhibition of phagocytosis by L.B.S. probably depends upon the absorption of some substance of the nature of complement—whether that substance be complement as usually understood or "non-immune opsonin" itself.

II.

Further Observations on Piantication.

Piantication of Bacillus pestis.—Having obtained the necessary immune sera, we proceeded to pianticate cultures of B. pestis, following the technique detailed in a previous publication by one of us.[2] In the course of this work we found that some further light was thrown upon the factors necessary for the accomplishment of piantication.

In order to pianticate a bacterium a considerable amount of preparatory work is usually necessary in order to obtain the immune serum. We find that a serum obtained from an animal which is developing marked antibacterial properties—as shown by agglutination and opsonic tests—is satisfactory.

Given the immune serum, some organisms can be quickly pianticated, provided that certain conditions are observed. We were successful, almost at once, in pianticating a strain of B. pestis by successive series of exposures to heated and unheated immune serum—the former consisting of immune serum which had been heated at a temperature of 56°C. for half an hour. The following table (VII.) records the progress of piantication as indicated by opsonic tests carried out with normal rabbit's serum; in these and all other similar tests the living organism was used:—

TABLE VII.

Pianticatiou of B. pestis by successive treatment with heated and unheated immune serum.

Date.	Immune Serum	Duration of Exposure.	Opsonic count with normal rabbit's serum.			
			Pianticated Strain of B. p		Original Strain of B. p. *	
			No. of Bacilli.	No. of Leucocytes.	No. of Bacilli.	No. of Leucocytes.
March 6	Heated	24 hours	—	—	—	—
,, 8	,,	96 ,,	27	25	3	25
,, 10	,,	24 ,,	8	25	7	25
,, 11	,,	6½ ,,	5	25	1	25
,, 12	,,	5½ ,,	22	25	1	25
,, 13	,,	6 ,,	24	25	9	25
,, 15	Unheated	24 ,,	109	25	9	25
,, 17	Heated	24 ,,	63	10	28	25
,, 19	,,	24 ,,	51	25	14	25
,, 20	Unheated	5 ,,	59	25	6	25
,, 22	,,	5½ ,,	90	25	19	25
,, 23	,,	5½ ,,	36	5	8	25
,, 24	,,	6 ,,	118	25	29	25
,, 25	,,	? ,,	87	25	0	25
,, 26	,,	8 ,,	41	10	1	25
,, 27	,,	7 ,,	42	5	7	25
,, 29	Heated	4 ,,	39	10	4	25

* Care was taken that the culture of the original organism was always of the same age as that of the pianticated organism: this was secured by subculturing both varieties simultaneously.

Relative Virulence of the Original and Pianticated Strains of B. pestis.

As our strain of B. pestis proved to be non-lethal, in moderate doses, for guinea-pigs, we were unable to make use of it in comparing the results of inoculations with the pianticated and the original strains. Another strain, obtained from Dr. Park Ross, of Durban, proved to be lethal for guinea-pigs, and with this we inoculated rabbits, and in a few weeks had obtained an immune serum; attempts to pianticate the B. pestis with this serum were, however, quite unsuccessful. A rabbit which had been inoculated with this same strain died from the infection; having recovered the organism from the heart-blood and spleen, we found that it, in its recently isolated condition, could be readily pianticated by alternate exposures to heated and unheated immune serum. The following phagocytic counts are taken from the record of our observations on this occasion:—

TABLE VIII.

Pianticated Strain.		Original Strain.	
No. of Bacilli.	No. of Leucocytes.	No. of Bacilli.	No. of Leucocytes.
51	25	0	25
110	50	7	50
60	25	14	25
79	25	10	25
124	25	6	25
81	25	2	25
112	25	6	25

A few inoculation experiments were now carried out with our virulent strain of B. pestis, in its original and its pianticated condition; these were undertaken with the view of ascertaining whether B. pestis when pianticated was less pathogenic than the original strain. Unfortunately we were not able to reach any definite conclusions on the point, as the supply of guinea-pigs became curtailed; owing to the war the preparation of anti-diphtheritic serum was undertaken at the Institute, with the result that nearly all the guinea-pigs were unexpectedly "commandeered." As far as our experiments went the results were as follows:—

TABLE IX.

B. pestis.—Original Strain.		B. pestis.—Pianticated Strain.	
	Result.		Result.
Guinea-pig inoculated subcutaneously with 20 millions.	Died in 5 days.	Guinea-pig inoculated subcutaneously with 200 millions.	Died in 6 days.
Rabbit inoculated subcutaneously with 20 millions.	Survived.	Rabbit inoculated subcutaneously with 200 millions.	Died in 6 days.
Rabbit and guinea-pig each inoculated subcutaneously with 200 millions.	Both died in 6 days.	Three guinea-pigs inoculated subcutaneously with 50 millions.	Died in 7 days.
		100 ,,	10 ,,
		200 ,,	6 ,,

When we sought to use white rats instead of guinea-pigs we found that they proved resistant to doses of up to 500 million bacilli: we therefore concluded that they were not suitable for our purpose.

Piantication of Bacillus coli communis.—We were equally successful in pianticating a strain of the B. coli communis, but in this instance the organism was exposed to heated serum alone. Table X. records the results obtained.

As we had no special reason for continuing to pianticate the B. coli communis, we merely re-planted the original strain from time to time. Some weeks later, however, we endeavoured to again pianticate the original strain, which had meanwhile undergone a number of sub-cultivations, but were quite unsuccessful. This strain had been obtained from the urine of a case of colon cystitis. At a later date another specimen of urine was sent to the laboratory from the same patient, and the bacillus was again isolated; we treated this freshly isolated bacillus and found that it was readily pianticated.

TABLE X.

Pianticatiou of B. coli communis by exposure to immune serum which had been heated to 56 C. for half an hour.

Date.	Duration of Exposure.	Opsonic count with normal rabbit's serum.			
		Pianticated Strain of B. coli c.		Original Strain of B. coli c.*	
		No. of Bacilli.	No. of Leucocytes.	No. of Bacilli.	No. of Leucocytes.
March 6	24 hours	22	25	12	25
,, 8	26 ,,	50	25	6	25
,, 10	24 ,,	3	25	1	25
,, 11	6½ ,,	20	25	29	25
,, 12	5½ ,,	29	25	17	25
,, 15	48 ,,	37	10	11	25
,, 17	24 ,,	50	5	10	25
,, 19	24 ,,	39	10	19	25
,, 22	5½ ,,	42	25	17	25
,, 25	— ,,	53	10	20	25
,, 26	8 ,,	36	5	10	25
,, 27	12 ,,	64	25	10	25
,, 29	4 ,,	42	25	8	25
,, 30	— ,,	40	10	23	25
,, 31	5½ ,,	61	5	9	25

* See footnote p. 9.

We had somewhat similar experiences with a Pasteurella organism; when attempts were made to pianticate it at a comparatively short interval after its isolation from the patient we were successful, and although similar attempts made some weeks later were ultimately successful a greater number of treatments were necessary. A third series of attempts at a still later date were quite unsuccessful. We

again obtained the organism from the patient—who by this time had greatly improved under vaccine treatment—but found that our attempts to pianticate it were now unavailing.

From these experiments we conclude that if a micro-organism is to be readily pianticated the following conditions are advantageous:—(1) The serum must be vigorously anti-bacterial; (2) the bacterium should have been recently isolated from the host; and (3) the latter should not have been submitted to vaccine treatment. If the host has been treated by vaccines, and thus rendered more or less immune, the bacterium has possibly had time to adjust itself to "immune" serum. This suggests the practical consideration, that when a patient is treated by a vaccine the first few doses are probably of pre-eminent importance. The infecting bacterium is not a passive intruder, it is a living parasite, and as such it is capable of adjusting itself to new conditions; from the point of view of successful therapeutics the attack upon it should therefore be as abrupt as possible.

III.

The Effect on Phagocytosis when the Serum in a Phagocytic Mixture is replaced by 1 per cent. Salt Solution.

In the course of our efforts to pianticate the B. pestis the observation was often made that phagocytosis occurred more readily when no serum had been added to the suspension of the organism in 1 per cent. saline. The record of a typical observation is as follows:—

TABLE XI.

Constituents of Phagocytic Mixture.	No. of Bacilli in 50 Leucocytes.
1 vol. suspension of B. pestis (original strain in 1% saline) + 1 vol. normal rabbit's serum + 1 vol. corpuscles.	63
1 vol. suspension of B. pestis (original strain in 1% saline) + 1 vol. 1% saline + 1 vol. corpuscles.	242

Similar results were also obtained with a diphtheroid bacillus which had been isolated from the nasal secretion of a patient.

From these observations it would seem that fresh serum may be less prejudicial to a bacillus—from the point of view of phagocytosis—than such an "indifferent" solution as 1 per cent. saline.

CONCLUSIONS.

(1) That if immune B. coli communis serum be incubated with its homologous organism certain properties are conferred upon it in virtue of which it is able, when mixed and incubated either with a similar immune serum or with a normal serum, to greatly inhibit the opsonic power of such sera towards Micrococcus pyogenes aureus. The effect is more marked when the mixture has been made with immune serum.

(2) That recent isolation of an organism is, in some instances, necessary if successful piantication is to be achieved.

(3) That it may be impossible to pianticate an organism if it has been isolated from a patient who has received active immunisation treatment against such organism.

(4) That some cultures of Bacillus pestis are more readily phagocyted when 1 per cent. saline is substituted for the serum of the phagocytic mixture.

(5) That the question of the relative virulence for guinea-pigs and rabbits of pianticated and ordinary Bacillus pestis is at present undecided.

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PROPHYLACTIC INOCULATION OF MAN AGAINST PNEUMOCOCCAL INFECTIONS, AND MORE PARTICULARLY AGAINST LOBAR PNEUMONIA; INCLUDING A REPORT UPON THE RESULTS OF THE EXPERIMENTAL INOCULATION, WITH A SPECIFIC GROUP VACCINE, OF THE NATIVE MINE LABOURERS EMPLOYED UPON THE PREMIER (DIAMOND) MINE AND THE CROWN (GOLD) MINES IN THE TRANSVAAL AND THE DE BEERS (DIAMOND) MINES AT KIMBERLEY—COVERING THE PERIOD FROM NOVEMBER 1, 1916, TO OCTOBER 31, 1917.

By F. S. LISTER, M.R.C.S., L.R.C.P.

During 1913 a classification of the Pneumococcus into groups which are biologically and immunologically distinct was accomplished by A. R. Dochez and L. J. Gillespie[1] in America, and independently by the Author[2] in South Africa. These discoveries have led to greatly increased precision in our conception of the immunological problems involved in pneumococcal infections.

Towards the end of 1915, and during the greater part of 1916, I carried out a laboratory investigation with respect to the possibility of conferring immunity against pneumococcal infections in the rabbit and in man. The results of this investigation convinced me that it was quite feasible to protect rabbits against experimental infections, and also that, in the case of man, the production of specific antibodies (agglutinins and opsonins) occurred regularly in response to inoculation with pneumococcal vaccine—provided that the dosage and the method of inoculation were suitable. I summed up (l.c.) the probabilities of success in the following words:—

"If the problem to be studied had been the immunisation of rabbits against pneumococcal infection one might say that it has been satisfactorily solved and the results put to a crucial test. I have shown that suitable inoculation will give rise in man to those antibodies which appear to be mainly responsible for the immunity which can be established in the rabbit . . . . it may be reasonably anticipated that

the resistance of an inoculated man to a natural infection by the Pneumococcus will be as satisfactory as that of the inoculated rabbit to an artificial infection—owing to the presence in his serum of the same demonstrable antibodies." "It is obviously of first importance that a suitable experiment should be organised to put this anticipation to the test. This can only be done by inoculating a group of individuals 'at risk' and recording the subsequent incidence of Pneumonia amongst them. In such an experiment it will be necessary to ascertain whether cases of Pneumonia which may arise amongst the inoculated are or are not due to organisms belonging to the groups comprising the vaccine; it would be manifestly illogical to expect protection against any group of Pneumococcus of the Lobar-Pneumonia-producing type which was not represented in the vaccine used."

Since the publication of the above, I have endeavoured to carry the work to the definite conclusion which I had suggested. Experiments on a large scale, dealing with the prophylactic inoculation of man against Lobar Pneumonia, have been conducted under the aegis of the South African Institute for Medical Research. In the following pages the results which have been obtained in these experiments are recorded, and such evidence as appears to be relevant to the issues under consideration is put forward.

The matter may be conveniently arranged under the following sections:—(I.) Nature of the evidence employed in evaluating the results. (II.) Preparation of the vaccine, dosage, and system of inoculation. (III.) The Premier Diamond Mine experiment. (IV.) The Crown Mines experiment. (V.) The De Beers Diamond Mines experiment. (VI.) Discussion. (VII.) Conclusions.

SECTION I.

Nature of the Evidence employed in Evaluating the Results.

The method of using controls in the conventional manner has been deliberately avoided in carrying out the experiments described in Sections III., IV. and V. This has been done, primarily, because the use of controls would have involved serious fallacies dependent upon certain of the epidemiological features of Lobar Pneumonia, and, secondarily, because of practical considerations which will be referred to presently in connection with two of the experimental mines. In lieu of a system of controls in these experiments, I have made use of a principle which has recently become available owing to the advances made in our knowledge of the bacteriology of the Pneumococcus. An application of this principle has for some time past seemed to me capable of affording very definite and irrefutable evidence as to the efficacy or otherwise of pneumococcal prophylactic inoculation.

A brief consideration may now be given to what appear to me to be certain fallacies which would have been inherent in the use of conventional controls in these particular experiments:—

In a previous publication[3] I expressed the following opinions:— "In the system hitherto employed on the Rand for assaying the results of prophylactic inoculation against the Pneumococcus a certain advantage conferred upon the uninoculated has, I think, been overlooked. If Pneumonia is spread, as I believe it to be, either directly from case to case or through the agency of carriers it follows that the inoculation of half the inhabitants of a Native compound may interrupt the chain, not only of actual pneumonic patients, but also of carriers; if the inoculation achieves this it is obvious that the uninoculated half of the population will reap an advantage which is not allowed for in the calculations."

Not only does the foregoing source of fallacy exist in an experiment controlled in the orthodox manner, but an additional one is also probably present. In view of the fact that the frequent passage of the Pneumococcus through laboratory animals increases its virulence to a quite remarkable degree it is highly probable that frequent passage through man produces a similar effect; may not pneumococcal prophylactic inoculation of one-half of a community (the members of which live and work together), by diminishing the number of passages of a given strain of Pneumococcus over any stated period, tend to keep its virulence at a lower level than would otherwise have been the case? If such were the result, then the uninoculated half of the community would be subjected to less risk than usual, whilst, on the other hand, the greater opportunity for passage of the Pneumococcus in the uninoculated group would tend to increase the risk to the inoculated.

When these possible sources of fallacy are considered we may well ask what justification there would have been in the present instance for assuming that all bias would have been eliminated, and errors of chance rendered computable, by recording the results obtained in a mixed population consisting of inoculated individuals and uninoculated controls.

At the time of the initiation of the present experiment on the Premier Diamond Mine Pneumonia was rife amongst the labourers, and it was sought to terminate such a state of affairs as early as possible; for this reason the whole Native labour force was at once inoculated, with the exception of a few Natives who, owing to the exigencies of the circumstances, were overlooked. Again, at the Crown Mines, for more than a year previous to the commencement of this experiment, the Native population had been inoculated as a routine measure, though the method which had been employed was not as efficient as that now under consideration. Uninoculated Natives already resident on these two mines at the commencement of the respective experimental periods were therefore not available as controls. Even if such resident Natives had not been inoculated a fallacy would have been introduced by employing them as controls— a fallacy which is based upon the fact that the mortality from Lobar

Pneumonia amongst Native mine labourers is twice or thrice as great during their first three months of residence on the mines as subsequently.

I was but little inclined to set aside a proportion of the new arrivals on these two mines as uninoculated controls, since, for reasons which I have stated, they would have reaped a very substantial benefit through association with such a thoroughly inoculated community.

The experiment at the De Beers Diamond Mines was commenced at a later date than those on the Premier and Crown Mines; the consistent results which were being obtained on both these latter mines, together with the fact that the period of service of Natives at De Beers is a very short one, induced me to adopt, on this mine also, the system of inoculating all fresh arrivals without any attempt to keep uninoculated controls.

Having now stated my reasons for having discarded the usual method of evaluation by comparing the incidence of and mortality from Lobar Pneumonia in inoculated and uninoculated (control) groups, it becomes necessary to state clearly the principles upon which, in my opinion, the organization of suitable experiments should be based, and the nature of the evidence on which I rely in forming an estimate of the results obtained. A satisfactory experiment should, I think, be governed by the following five conditions:—

(I). The inoculated population should be large;

(II). The experiment should extend over a lengthy period;

(III). A bacteriological and serological investigation should be carried out in every case of Lobar Pneumonia occurring in the inoculated population during the experimental period;

(IV). A comparison should be made between the rates of incidence of and, more especially, mortality from, Lobar Pneumonia in the selected population during the experimental period and the same rates in the same population over a lengthy period previous to inoculation;

(V). The inoculation and medical history of every inoculated person should be accurately recorded and kept by the card system.

With regard to conditions "I." and "II." little need be said; it is obvious that the larger the experimental group, and the longer the period of observation, the less is the chance that fortuitous circumstances will interfere with a correct interpretation of the results.

The importance and reliability of the evidence obtainable from the investigation demanded by condition "III." can scarcely be exaggerated. With our present knowledge we can determine, with a very small margin of error, to which group of the Pneumococcus the

infection in any case of Lobar Pneumonia is attributable. Between 63 and 76 per cent. of all cases of Lobar Pneumonia amongst Native mine labourers is caused by the Pneumococcus belonging to one or other of the groups "A," "B," or "C"; if the inoculation of a large group of labourers with a vaccine comprising these three prevalent groups greatly decreases the frequency of their occurrence, relatively to other pneumococcal groups, the result should constitute very weighty evidence in favour of inoculation.

The premisses upon which the above argument rests will not be contested by anyone who has personally investigated the question. The existence of definite groups of the Pneumococcus, distinct and possessing specific immunity reactions, has been placed beyond all doubt; the results of the earlier workers in this field have been confirmed in many laboratories, whilst no evidence has been advanced against their position from any quarter.

The relative frequency with which the various groups of the Pneumococcus have been found by me in 148 unselected cases of Lobar Pneumonia amongst Native mine labourers is shown in Table I. It may be remarked that none of the cases in this table were inoculated Natives on the three experimental mines. During the present year (1917) I have discovered three new and apparently important groups of the Pneumococcus, which I have designated "H," "J" and "K" respectively; several cases and deaths attributable to these three new groups have occurred amongst the inoculated Natives on the Crown Mines, but they do not appear in Table I.

Table I. shows that the Pneumococcus of groups "A," "B" and "C" occurred collectively in from about 77% (1914) down to about 63% (1917) of the cases, with an average for the five years of 68·9%. The comparatively high figure for group "B" in 1916 was due to the fact that an outbreak at the Premier Diamond Mine was investigated, when in seven consecutive cases the Pneumococcus of this group was found to be the infecting organism*; A. R. Dochez, of the Rockefeller Institute, has informed me that of 495 cases of Pneumonia, 60 per cent. was found to be due to the Pneumococcus of types I. and II.; these two types are identical with my groups "C" and "B" respectively. Group "A" Pneumococcus, which is the predominant group amongst the Natives of the Transvaal, appears to be unknown in America.

I need not further labour the point that in Natives employed in the mines of the Transvaal Lobar Pneumonia is consistently due, in the majority of cases, to infection by the Pneumococcus of groups "A," "B" or "C"; if prophylactic inoculation against these three groups, only, entirely alters this epidemiological peculiarity, the procedure will practically rank as a crucial experiment—an experiment which should be free from statistical criticism if the data furnished

* On another occasion an epidemic of Pneumonia due to group "A" Pneumococcus occurred on one of the gold mines.

TABLE I.

The different groups of the Pneumonia-producing Pneumococcus, and the number of individuals infected by each group, discovered during five periods of investigation amongst uninoculated native labourers on various mines from 1913 to 1917, inclusive.

Groups.	1913.	1914.	1915.	1916.	1917.	Totals.	% of Totals.
"A"	9	12	16	4	5	46	31·0
"B"	5	5	1	10	3	24	16·2
"C"	1	3	15	4	9	32	21·6
"D"	3	3	0	0	0	6	4·0
"E"	0	2	0	0	0	2	1·3
"F"	0	0	7	1	1	9	6·0
"G"	0	0	4	6	1	11	7·4
"H"	0	0	0	0	4	4	2·7
"J"	0	0	0	0	1	1	·6
"K"	0	0	0	0	1	1	·6
"X"	0	0	6	0	1	7	4·7
"Unclassifiable" ...	2	1	1	0	1	5	3·3
No. of Specimens ...	20	26	50	25	27	148	

are sufficiently ample and the results sufficiently pronounced. It is not my intention to proclaim any numerical standard which is to be accepted as final proof of the efficacy of prophylactic inoculation against Lobar Pneumonia; there are individuals who are hardly to be convinced in any circumstances, whilst others are prepared to accept as proof evidence of the slenderest character. To my mind, the results obtained by this particular method of investigation would amount to practical failure if they were so unapparent as to require biometrical analysis to disclose them; so deeply hidden a fruit might have some academic value, or might prove a stimulus to further research, but viewed from the utilitarian point of view the harvest would be a failure. It is, then, upon this method, and the results obtained by it in connection with the Crown Mines experiment, that I desire to lay particular emphasis, for these results, in my opinion, irrefutably demonstrate the practical value of prophylactic inoculation.

The class of evidence demanded by condition "IV." needs but little elaboration; why the statistics of any given mine during the experimental period should be compared with those of *the same mine* at other periods requires, however, some explanation. Both the incidence of and mortality from Lobar Pneumonia differ very widely on different mines of the Witwatersrand; on some they have always been high, whilst on others the reverse has obtained—a discrepancy which was also apparent during the period when "Tropical"* Natives were employed. Owing to this pecularity it is obvious that statistical arguments either favourable or unfavourable to prophylactic procedures can be produced at pleasure if licence be given to select mines for the purposes of comparison.

The three mines chosen for the carrying out of the experiments here dealt with had always experienced, prior to inoculation, a very heavy mortality from Lobar Pneumonia, and the Crown Mines had been one of the worst on the Witwatersrand in this respect; even the high Pneumonia death-rate occurring on this gold mine, however, had been greatly exceeded on both the Premier and the De Beer's Diamond Mines for several years previously. If a substantial reduction of the Pneumonia death-rate—*e.g.*, a reduction to below that for the same period for all Native labourers in the proclaimed labour area—were to occur simultaneously on each of these three mines coincidently with inoculation, it would be justifiable to regard the occurrence as valuable collateral evidence in favour of prophylactic inoculation. Such evidence would be, of course, of quite another order to that required by condition "III.," and would not carry so much conviction, at any rate to the Immunologist.

All that is necessary to state with regard to the fifth condition is that it was fulfilled.

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* "Tropical" Natives are those who have been recruited from territories to the north of latitude 22°S. Their further recruitment for labour in the mines of the Witwatersrand was prohibited by the Union Legislature in May, 1913.

Owing to its accessibility, the Crown Mines was especially selected for the investigation stipulated for in condition "III." and, with two exceptions,* every case of Lobar Pneumonia which occurred amongst its inoculated population during the experimental period of nine months was made the subject of a detailed bacteriological and serological investigation. This investigation comprised:—

1. Tests of the patient's pre-critical serum with stock cultures of the groups "A," "B" and "C"—to determine the immunity-response to his previous prophylactic inoculation.
2. Isolation of his infecting organism—by means of mouse-inoculation with his sputum.
3. Identification of the group of Pneumococcus to which the isolated organism belonged—by means of agglutination tests performed with the various stock antisera specific for different groups.
4. Test of patient's post-critical serum—to confirm, or otherwise, the findings obtained with the organism isolated from his case. For this purpose the serum was tested with a representative stock culture of each of my established groups, and also with the patient's homologous organism.
5. In all cases that ended fatally cultures were obtained direct from the lung, by mouse-inoculation, and were tested for group identification by means of stock antisera specific for various groups.

An effort was made, for a time, to carry out the same investigations in connection with the other two experimental mines, but owing to their distance from Johannesburg, and the time occupied in the transmission of specimens (with their consequent decomposition), the work was found to be impracticable and was abandoned. The evidence to be submitted with regard to the Premier and the De Beers mines is therefore mainly that required by condition "IV." Finally, it should be pointed out that I have not been responsible for the clinical diagnosis of any of the cases which occurred on the three mines during the experimental periods.

SECTION II.

Preparation of the Vaccine, Dosage, and System of Inoculation.

The rationale of designing a vaccine for the prophylaxis of Lobar Pneumonia can only be appreciated by those who are familiar with the more recently discovered facts concerning the epidemiology of this disease. Too much space would be occupied here by a detailed exposition, and as numerous publications dealing with this question have appeared during the last four years it must be taken for granted that those interested in this subject are conversant with the details.

* One of these cases occurred during my absence on Pneumonia investigation at Kimberley. The other was that of a Native who had been isolated as a suspected case of Typhus; it was only after his recovery, and upon his discharge, that the diagnosis of Lobar Pneumonia was decided upon.

The collecting of immunologically distinct strains of the Pneumococcus is an essential preliminary to the preparation of a suitable vaccine, and their differentiation and identification can be achieved by the procedures which I have published elsewhere.[2] The decision as to what groups are to be represented in the vaccine depends upon the relative prevalence of the various groups amongst the population to be protected; this relative prevalence can only be determined by careful laboratory investigation of many local cases of pneumococcal infection.

To obtain sufficient culture for the vaccine it is not practicable to make use of solid media, owing to the great quantity of it which would be required and the relatively poor growth of the Pneumococcus in such circumstances.

After considerable investigation a fluid medium has been obtained in which the Pneumococcus grows *profusely*, this qualification being intended to imply a growth of from three thousand to three thousand five hundred millions of cocci per c.c. The medium is essentially Douglas's trypsinated bullock's heart medium,[4] to which has been added small quantities of sodium phosphate and glucose.

The reaction necessary for the optimum growth of the organism has been found to differ very widely from that advised by other workers; I have found the most favourable reaction to be +17 (per litre) to phenol-phthalein—the titration being performed at the boiling point according to Eyre's method. To what extent the newer conceptions of "Hydrogen ion concentration" and "buffer content" of the medium are concerned I am not in a position to state.

The directions for the preparation of the medium I employ are as follows:—Cut off the fat and large vessels from an average-sized, fresh bullock's heart; mince; add 4 litres of water and 4 grammes of dibasic sodium phosphate; make fairly alkaline to litmus; heat to between 70° and 80° C.; cool to 45° C., and add 1 per cent. "Liquor Trypsin Co." (Allen and Hanbury); place in the incubator at 37° C. for about three hours, but on no account allow the action of the ferment to proceed too far; acidify with acetic acid, and boil for half an hour; strain, and adjust the reaction to +17 per litre (phenol-phthalein) by addition of dekanormal caustic soda; add ·013 per cent. of calcium chloride, ·2 per cent. of glucose, and ·25 per cent. of sodium chloride; autoclave for half an hour at 125° C.; filter into wide-mouthed culture-bottles, and finally sterilize in the autoclave for half an hour at 120° C.

It is to be especially noted that no fresh blood, or serum, is subsequently added to this medium; it may, however, be remarked that the pneumococcal strains which I now employ have been long isolated, and, therefore, perhaps, grow more readily than if they had been recently isolated.

When growth of the Pneumococcus has occurred in this medium, autolytic changes rapidly take place; after twelve hours' incubation a close watch must therefore be kept, or the growth may speedily be rendered useless for the preparation of vaccine.

Sub-cultivation of the strains which are employed for the vaccine should be maintained on ordinary blood-agar; continued subculture in the trypsinated medium is difficult, and I have some evidence that, with certain strains at least, the trait of agglutinability disappears in such circumstances.

In previous attempts at pneumococcal prophylactic inoculation in South Africa (and I refer more particularly to the work of Sir Almroth Wright and his associates) the entire broth culture of the Pneumococcus was used. The vaccine which has been used in these experiments consists of a suspension of the bacterial bodies in normal saline (·85 per cent.) the bacterial bodies having been recovered from the broth culture by centrifugalization.

The lack of a rapid and practical method of recovering the cocci from the broth culture was, at the outset, a serious obstacle to preparing the vaccine in large quantities, but this difficulty was eventually overcome; as much as 20 litres of broth culture are now used as a daily routine, and occasionally 40 litres have been dealt with. A short experience showed us that the ordinary bucket-centrifuge was quite impracticable for our purpose. After experimenting unsuccessfully in many directions, I ultimately found that a particular type of cream-separator was capable of very efficiently retaining the cocci in the revolving bowl of the machine, the broth being, of course, ejected. The machine which I am now using is known as "The Pera," and has a bowl fitted with an arrangement of metal leaves; it has been adapted to run by means of an electric motor, and its performance gives great satisfaction. That type of machine in which the bowl is fitted with superimposed metal cones has proved useless.

The viscosity of the culture fluid is probably an important factor in successfully separating the organisms by centrifugal force. Now that trypsinated broth is employed, I find it necessary to dilute the grown culture with an equal quantity of sterilized water if the cocci are to be retained in the bowl; this necessity did not exist when ordinary peptone broth with added blood or serum was used.

The concentrated suspension of Pneumococci obtained from the bowl usually contains from twenty to forty thousand millions of cocci per c.c.

Sterilization of the suspension of Pneumococci is effected by the addition of an antiseptic and not by heat. As might be inferred from a consideration of this method of preparation, a few spores of the B. subtilis are constantly present in the suspension, and they are not easily killed. I find that complete sterilization of the suspension by the addition of 1·5 per cent. of carbolic acid, or even 2 per cent. of Tricresol, is usually not accomplished at room temperature until after the lapse of several weeks; by the use of "Medical Bacterol" (Menley and James) in the proportion of 1·5 per cent. it is, however, possible to sterilize all resistant spores in about five days. It is perhaps needless to say that the complete sterility of the suspension is ascertained by rigid tests before it is employed in the final make-up of the vaccine.

The particular strains of the Pneumococcus which have been used for the preparation of the vaccine employed in the three experiments were all isolated from cases of Lobar Pneumonia, and are now of considerable laboratory age; this is shown in the following Table:—

TABLE II.

The various Lengths of Time that have elapsed since the vaccine Strains were isolated.

Strain.	Group.	Period elapsed since isolation.
2,480	"A"	Two years and one month.
3,915	"B"	One year and one month.
3,472	"C"	Two years and one month.
209	"H"	Eight months.

These strains have not been subjected to animal passage; they retain good antigenic qualities, and are readily agglutinated by their homologous group antisera, and by no other.

In a previous publication[3] I illustrated in detail the antibody production in the sera of the rabbit, and man, which is consequent upon their inoculation with a vaccine containing representatives of *five* distinct groups of the Pneumococcus; and I advised that—"For prophylactic purposes in man the subcutaneous method should be adopted. Three inoculations, at seven days' interval, should be employed; each dose should consist of 6,000 (six thousand) million cocci of each group against which immunity is desired." The vaccine employed in the present experiments did not comprise representatives of five groups, and the dose administered did not contain six thousand millions of cocci of each group; the subcutaneous inoculation of three doses at seven days' interval was, however, adhered to. An explanation of these apparent inconsistencies, and the reasons for departing from some of my former conclusions, may now be stated.

My experimental study of prophylactic inoculation against pneumococcal infections was concluded in August, 1916. Almost immediately after this, the military authorities requested the South African Institute for Medical Research to furnish, at very short notice, some forty thousand doses of a pneumococcal vaccine for the immunization of the Natives about to proceed to France as a Labour Contingent.* The

* The results of this immunization appear to have been very satisfactory; they are not detailed in the present review as complete official figures are not yet available.

rapid preparation of so large a quantity of vaccine, comprising five groups and containing a total of thirty thousand millions of cocci in each dose, was, at that time, out of the question. I therefore advised the issue of a vaccine containing representatives of groups "A," "B," and "C" only, with a total content of ten thousand millions of cocci per dose of 1 c.c. Subsequently (November, 1916) a vaccine which was similar to this, in so far as the groups of the Pneumococcus are concerned, was employed on the Premier Diamond Mine; this vaccine, however, contained a still smaller number of bacterial bodies, viz., a total of seven thousand millions per dose of 1 c.c. In Section "III." it will be shown that the epidemic prevailing on the Premier Mine at the time was at once suppressed by the use of this vaccine, and that from that time onwards (a period of one year) Lobar Pneumonia has ceased to give cause for any anxiety. When the experiment on the Crown Mines was started three months later (February 1, 1917) one was naturally influenced by the Premier Mine results, and as the preparation of a large quantity of vaccine was now necessary, I decided to use a vaccine similar to that which had been adopted on the Premier Mine. At a still later date (May 1, 1917) the De Beers Diamond Mines experiment was undertaken. For reasons referred to in Section "V," I made certain alterations in the vaccine for use on this mine, the alterations consisting in the addition of a representative of another group ("H") and an increase in the total number of cocci to eight thousand millions per dose of 1 c.c. I find that doses of this richness (two thousand millions of each group) give rise to demonstrable antibodies in the sera of the majority of those inoculated; the antibody production is, however, less marked and less consistently uniform than when the larger doses originally suggested by me are employed.

It will thus be seen that the practical difficulties in the way of preparing the vaccine in large quantities on the lines originally recommended led me, in these experiments, to test its efficacy when modified both in respect to the number of pneumococcal groups employed and also the richness of the dose.

In carrying out these experiments it was decided to inoculate only the new arrivals on the selected mines. The average length of stay of a Native on the gold mines is about eight months, and on the diamond mines a still shorter period; under this system, therefore, a substantial proportion of the Natives will have undergone inoculation in the course of a few months, and this will have been accomplished without arousing the antagonism of those Natives already resident on the mines at the initiation of the experiment. A still weightier reason for the adoption of this system is the necessity for immunizing all Natives as soon as possible after their arrival, on account of their far greater liability to contract Pneumonia during their first three months of service. I am of the opinion that if Pneumonia is prevented amongst the new arrivals, considerable benefit accrues to the uninoculated portion of the population. On the Premier Diamond Mine, however, the whole Native population, as stated above, was at once inoculated, whilst the Natives resident on the Crown Mines at the initiation of the experiment had been inoculated previously.

Except for the preliminary mass inoculations carried out on these two mines, the system outlined above, *i.e.*, the triple inoculation of every fresh arrival, has been consistently employed on each of the three experimental mines from the beginning of their respective experimental periods.

The duration of the experimental periods was as follows: Premier Diamond Mine, 12 months; Crown Mines, 9 months; De Beers Diamond Mines, 6 months.

To recapitulate: The vaccine used in these three experiments consisted of the bacterial bodies from a young broth culture, suspended in normal saline; sterilization was effected, without heat, by the addition of an antiseptic. In the case of the Premier and Crown Mines, the vaccine used contained a representative of each of the groups "A," "B," and "C" only, whilst at De Beers a representative of group "H" was also included.

SECTION III.

The Premier Diamond Mine Experiment.

The Premier Diamond Mine is situated about forty-five miles north-east of Johannesburg, and is an open working. Pneumonia has always been notoriously rife and a serious cause of mortality among its Native employees. The author was Assistant Medical Officer of this mine from August, 1907, to February, 1912, and is therefore well acquainted with the conditions. Inoculation against Pneumonia was resorted to in 1912, during Sir Almroth Wright's visit to the Transvaal. A portion only of the population was at first treated, but subsequently the whole Native labour force was inoculated, and the death-rate from Lobar Pneumonia dropped from 19·26 per thousand per annum, in 1912, to 4·8 per thousand per annum, in 1913. Much criticism was levelled at the published results obtained on this mine during that period, criticism which was chiefly directed against the alleged (though obviously unintentional) *selection* of the controls; I did not, at the time, acquiesce in these strictures, and am still quite unable to account for the satisfactory decline in the Pneumonia returns on any other grounds than that it was the direct consequence of the inoculation.

The Pneumococcus, however, had not, at that time, been classified into distinct groups, and it is therefore impossible to know either what groups were prevalent in the community inoculated or what groups were represented in Sir Almroth Wright's vaccine. It is, presumably, permissible to infer that the Pneumococcus of groups "A," "B," and "C" was predominant in the affected population. The composition of the vaccine is, however, less reasonably surmised, because, although nominally polyvalent, it is not in the least probable that the number of organisms of each group was in due proportion, even if, as seems improbable, all the leading groups were represented; that an efficient dose of *each* leading pneumococcal group was actually present is still less likely, seeing that the total dose inoculated only

contained one thousand million cocci. It may nevertheless be reasonably surmised that, in this particular instance, the vaccine strains corresponded with some of those causing infection in this mining population.

Similarly satisfactory results were, unfortunately, unobtainable when the same vaccine was very extensively employed on the gold mines of the Witwatersrand, and the systematic inoculation of the Natives on these fields was therefore temporarily abandoned. The Premier Mine alone, of all the mines in the Transvaal, continued to employ this means of Pneumonia prophylaxis.

Owing to the war, work on the Premier Mine was suspended in 1914, and operations were not resumed on any large scale until about August, 1916. With the increase in the number of Natives, from this month onward, Pneumonia again became rife, and during September and October the disease began once more to assume epidemic proportions. The returns relating to these two months were as follows:—

TABLE III.

Premier Diamond Mine, Pneumonia Returns for September and October, 1916.

	Average number of Natives employed.	Number of Pneumonia cases.	Number of Pneumonia deaths.
September, 1916	6,378	55	4
October, ,,	6,737	41	13

I investigated seven of the cases which had been admitted to hospital during October, and found that Pneumococcus of group " B " was the infecting organism in all of them.

At the beginning of November, Dr. A. J. McClymont, the Medical Officer of the Mine, inoculated, with a very few exceptions, all the Natives resident on the property, using for this purpose a vaccine containing groups " A," " B," and " C," and having a total content of four thousand millions of cocci per dose. These Natives received only two inoculations of this particular vaccine, whilst all new arrivals, from this time onward, were inoculated subcutaneously, on three occasions, at seven days' interval, with " A," " B," and " C " vaccine in the increased dose of seven thousand millions of cocci. The epidemic ceased immediately, and the cases and deaths from Pneumonia during November were three and one* respectively. These, then, were the circumstances in which the present experiment was inaugurated, an

* An additional death occurred in November of a Native, included in the above 41 *October* admissions, making the total of deaths 14. I have not included this one case in the statistics relating to the experimental period.

experiment which has been carried on for a period of twelve months. The following table shows the Pneumonia returns from the mine for a number of years, and also those for the experimental period November, 1916—October, 1917, inclusive.

TABLE IV.

Premier Diamond Mine, Pneumonia Returns for the Years 1908-1913, inclusive, together with those of the Experimental Period November, 1916—October, 1917, inclusive.

Year.	Pneumonia incidence per thousand per ann.	Pneumonia mortality per thousand per ann.
1908	70·71	14·17
1909	104·88	17·97
1910	153·54	28·97
1911	128·98	26·94
1912*	67·68	19·96
1913*	31·75	4·80
1914 } 1915 }	Returns unavailable owing to interruption of mining by the war.	
Nov. 1916—Oct. 1917 (Experimental period)	4·67	0·86

* During these years inoculation was carried out in accordance with the principles advocated by Sir Almroth Wright and his associates.

During the period November, 1916—October, 1917, inclusive, there were 10,507 new arrivals, and the mean population was 5,621. For all practical purposes the entire population over the whole period was a thoroughly inoculated one. During this period there occurred but seven cases of Lobar Pneumonia, with one death, in Natives who had received their full number (three) of inoculations.

The returns for this period, as shown in Table IV., are very striking, even when compared with those for the best previous year in the history of this mine, viz., 1913, when thorough inoculation had been secured with Sir Almroth Wright's vaccine. Owing to the latter circumstance it would be manifestly illogical to base conclusions as to the efficacy of the present method on a comparison with the returns for 1913; a comparison should rather be made with the figures relating to the years 1908-1911, inclusive. It should, however, be pointed out that a certain number of "Tropical" Natives were employed during part of the latter period, and it is well known that the Pneumonia mortality amongst such labourers is much higher than that amongst "Non-tropicals"; the ratio of "Tropical" employees to the whole labour force was, however, so small that their greater susceptibility did not materially influence the gross mortality from the disease.

In assessing the results of this experiment it is especially necessary to bear in mind the position with regard to Pneumonia in the months of September and October, 1916, immediately preceding inoculation.

The evidence which is relied upon in connection with the experiment on this mine is that required by No. IV. of the "conditions" postulated, in Section I., for the conduct of a satisfactory experiment; valuable as such evidence is, it is nevertheless not so convincing as that now to be described in connection with the experiment on the Crown Mines.

SECTION IV.

The Crown Mines Experiment.

This mine is situated in Johannesburg, and is one of the largest of the gold mines of South Africa as regards its complement of Native labour; when such labour is freely procurable, some fourteen to sixteen thousand Natives are resident in its compounds. This population is a floating one; the average length of service in the case of Portuguese East African Natives is about one year, and in that of all other tribes about six months. The proportion of Portuguese Natives to the total Native labour force is constantly about one-third.

The relative incidence of and mortality from Pneumonia on this property for the three years which preceded systematic inoculation in 1916 exceeded the average rates for all the Natives of the Witwatersrand gold mines,* so that its past history in this respect may be looked upon as distinctly bad. The Annual Report for 1916 of the Chief Medical Officer (Dr. H. A. Loeser) shows that the mean annual death-rate from Lobar Pneumonia on this mine for the period 1910-1914, inclusive, was 6·75 per thousand amongst all Natives recruited from south of 22° S., and 9·91 if the mortality amongst the "Tropical" Natives be included.

Notwithstanding the fact that the last "Tropical" Natives arrived on the mine in 1914,† it is remarkable that the returns from this mine for 1915 show a higher rate of mortality from Lobar Pneumonia amongst Natives from south of 22° S. than in any previous year, whilst the few remaining "Tropical" Natives, on the other hand, experienced a death-rate of only about one-third of that prevailing amongst their usually less susceptible associates.

In this report Dr. Loeser states:—"In view of the very great prevalence of Lobar Pneumonia amongst the coloured labourers

* For the three years 1913-1915 the mean annual death-rate from Lobar Pneumonia for all Native labourers on mines and works in the Transvaal was 6·16 per thousand, and 8·72 for those employed by the Crown Mines—deaths occurring amongst "Tropical" Natives being included in both instances.

† In May, 1913, the further recruiting of "Tropical" Natives was prohibited by law on account of their high death-rate, particularly from Lobar Pneumonia; outstanding contracts for labour were, however, permitted to be fulfilled.

on the Crown Mines at all times, and particularly in the latter part of 1915 . . . I decided to introduce prophylactic inoculation on a large scale, and this was begun at the end of October, 1915. Lister's Polyvalent Pneumococcal Vaccine, supplied by the S.A. Institute for Medical Research, was used in a single dose of 2,000 million organisms. New boys were inoculated on arrival and the rest of the complement gradually. By the beginning of April, 1916, all boys had been inoculated, and they were re-inoculated every three to four months. Altogether 64,000 inoculations were carried out between the end of October, 1915, and the end of December, 1916." Further on he states: "The reduction in incidence rate . . . and case mortality has run parallel with preventive inoculation and improved nursing. Whether there is a causal relation is matter for speculation."

The reduction to which Dr. Loeser refers is illustrated in Table V., which gives the returns for Pneumonia on the Crown Mines for a number of years. The marked improvement in the Pneumonia death-rate for the year 1916, when inoculation was systematically practised, is every obvious.

TABLE V.

Crown Mines, Pneumonia Returns (exclusive of "Tropical" Natives) for the years 1910-1916, inclusive, together with similar Returns for the Experimental Period February 1st—October 31st, 1917.

Year.	Pneumonia incidence-rate per thousand per ann.	Pneumonia death-rate per thousand per annum.	Case mortality per cent.
1910	34·04	7·37	21·66
1911	33·78	6·44	19·07
1912 (a)	23·20	5·78	24·90
1913 (b)	28·86	7·64	26·49
1914 (c)	26·48	6·60	24·92
1915	41·17	8·33	20·23
1916 (d)	24·82	4·01	16·15
Feb. 1st to Oct. 31st, 1917	13·89	2·95	21·21

(a) A large number of "Tropical" Natives were inoculated during this year by Sir Almroth Wright.

(b) Approximately three-fifths of the Portuguese East African Natives and British Basutos *resident on the mine* were inoculated with Sir Almroth Wright's vaccine.

(c) Over a period of four months *every alternate new arrival* was inoculated once with a specific group vaccine (Lister) consisting, in this instance, of a broth culture containing five specific groups of the Pneumococcus, with a total content of 2,000 millions of organisms per dose. A statistical criticism of the results of this experiment was published.[5]

(d) The whole Native labour force was systematically inoculated throughout this year with "Lister's Polyvalent Vaccine," which in this instance consisted of a suspension of bacterial bodies in normal saline, five specific pneumococcal groups being employed, and the total content being 2,000 millions of organisms per dose.

Such then had been the circumstances with regard to the prevalence of Pneumonia at the time of the initiation of this experiment on February the 1st, 1917. From this date all *new arrivals* were inoculated thrice with the vaccine and by the method described in Section II.; the experiment extended over a period of nine months. The total number of new arrivals during the nine months was 10,866, and of these 3,007 had again left the mine before the end of the experiment; such departures were naturally more numerous during the later months of the experimental period owing to the expiration of the contracts of service.

From Table V. it will be seen that the case mortality was higher for the experimental period than for the preceding year; this is attributable to the high case mortality which prevailed amongst the Natives who were already resident on the property previous to the initiation of the experiment. That this explanation is valid becomes apparent when we analyse the returns for the two groups, as follows:—

TABLE VI.

	No. of cases of Lobar Pneumonia.	No. of deaths from Lobar Pneumonia.	Case mortality per cent.
Natives not belonging to experimental group ...	49	17	34·7
Natives belonging to experimental group ...	82	12	14·63

The mine is easily accessible from the Institute, and I was able to visit it daily; these favourable circumstances permitted the systematic carrying out of a laboratory-controlled investigation over the whole period, thus fulfilling the requirements of Clause III. in my scheme of "satisfactory conditions" detailed in Section I. In Table VII. will be found an analysis of all the cases of Lobar Pneumonia which occurred amongst the inoculated population, showing the number of cases associated with the various pneumococcal groups. The total number of cases was 82, and of these 12 terminated fatally.

TABLE VII.

Distribution of the various Groups of the Pneumococcus in 82 consecutive Cases of Lobar Pneumonia amongst the Natives on the Crown Mines who had been inoculated during the Experimental Period (Feb. 1st to Oct. 31st, 1917.)

	"A"	"B"	"C"	"D"	"E"	"F"	"G"	"H"	"J"	"K"	X	Unclassifiable.	Not Investigated.	Totals.
All cases	0	0	0	1	14*	6	3	13	2	4	7	30	2	82
Fatal cases	0	0	0	0	3	0	0	2	2	3	0	2†		12

* The post-critical serum of five of these cases, all of which ended in recovery, failed to agglutinate the group "E" pneumococcus which had been obtained from the sputum; this organism was therefore probably merely a saprophytic inhabitant of the upper air passages in these particular patients, and had no causal relation with their disease.

† Not investigated as regards groups "J" and "K."

The method employed for the purpose of group identification of the strains is sufficiently detailed in Section I.

It may be remarked that those cases of Lobar Pneumonia which ended in recovery yielded a larger number of unclassifiable strains of the Pneumococcus than I had anticipated. The symptoms associated with these infections were usually extremely mild; on the other hand, ten of the twelve cases which terminated fatally were due to infection by classifiable strains, and the remaining two, shown in the table as "unclassifiable," had unfortunately been lost before I had discovered the existence of the groups "J" and "K"; the opportunity of identifying these two cases with either one or the other of these new groups had not, therefore, presented itself. It is significant that all of five strains which I recovered from fatal cases of Pneumonia, and which would have been "unclassifiable" prior to my discovery of these two groups, were at once found to belong to either one or the other of them.

The most striking fact disclosed in Table VII. is the entire absence of any representative of groups "A," "B," or "C" amongst this large number of consecutive cases of Lobar Pneumonia; as previously stated, I have definitely established the fact that, under normal circumstances, these three groups are together responsible for from 63 to 77 per cent. of all cases of Lobar Pneumonia in the Native mine labourers of South Africa. Since this particular population had been inoculated with a vaccine containing only the Pneumococcus of these three groups I have no hesitation in maintaining that the inoculation stands in direct causal relation to the results. The strict immunological specificity of these pneumococcal groups is also further confirmed by these results.

It would, of course, be inaccurate to say that at least 60 per cent. of these 80 *investigated* cases would, but for the inoculation, have been due to groups "A," "B," and "C" collectively. The correct way of estimating the probabilities is, I think, to assume that, since some 40 Pneumonias due to strains other than "A," "B," and "C" are found in normal circumstances to be associated with some 60 other cases due to "A," "B" and "C" collectively, about 120 cases of "A," "B," and "C" Pneumonia would have occurred amongst these inoculated Natives during the nine months.

An inspection of the investigation results for 1917, in Table I., shows that Pneumonia due to groups "A," "B" and "C" was actually prevalent amongst (uninoculated) Natives on various mines during the time that the experiment on the Crown Mines was in progress, and, furthermore, that it constituted about 63 per cent. of the cases investigated.

I had not anticipated so complete an abolition of "A," "B," and "C" Pneumonia, because the Natives had not received their first inoculation until *after* their arrival at the mine, although usually within

twenty-four hours of that event. As there were 10,866 fresh arrivals during the experimental period it is more than likely that a few would have arrived during the incubation period of their infection by these strains; either this was not the case, or their first inoculation aborted the impending attack.

Such a sequence of cases of Lobar Pneumonia in Natives without the occurrence of a single case due to Pneumococcus "A," "B" or "C" must be unique, and would probably never occur in the absence of this specific prophylactic factor. The results of this experiment alone constitute, to my mind, a proof of the efficacy of this particular method of prophylactic inoculation against Lobar Pneumonia.

Statistical investigations as to the value of preventive inoculation against Lobar Pneumonia have hitherto been based upon the assumption of the oneness of the Pneumococcus which causes this disease. If this assumption were to be made in dealing with the present returns from the Premier and the Crown Mines it is evident that the conclusions arrived at would be entirely erroneous, for the fact of the complete abolition from the Crown Mines of Pneumonia due to the vaccine groups would not be disclosed. It is evident that full use must be made of the discriminations made possible by recent serological advances whenever statistical comparisons are attempted in the domain of preventive medicine.

SECTION V.

The De Beers Diamond Mines Experiment.

The De Beers Mines are situated at Kimberley, in the Cape Province, about three hundred miles from Johannesburg. The number of Natives employed, in ordinary times, is about 13,000; at present only about 7,400 are working on those of the De Beers Mines which are included in this experiment.

As with the Premier Diamond Mine, so here also, Lobar Pneumonia has been a scourge for a number of years. The following table sufficiently indicates the prevalence of the disease:—

TABLE VIII.

The De Beers Diamond Mines, Pneumonia Returns for the Years 1908-1914, inclusive.

Year.	Pneumonia incidence per thousand per annum.		Pneumonia mortality per thousand per annum.		Case mortality per cent.	
	Underground.	Surface.	Underground.	Surface.	Underground.	Surface.
1908	37·05	6·12	7·57	2·04	20·42	33·33
1909	69·52	8·65	12·45	1·50	17·91	17·39
1910	84·50	31·68	19·27	1·94	22·81	6·13
1911	83·63	16·55	16·39	2·82	19·60	17·02
1912	61·51	15·98	14·40	·68	23·41	4·24
1913	—	—	13·41	1·94	—	—
1914	Figures for complete year not available owing to interruption of mining by the war.*					

* The Pneumonia returns for this year, up to the time of the interruption of mining, in September, were considerably less favourable than those for 1913.

The returns given in the preceding table indicate a far greater incidence of and mortality from Lobar Pneumonia amongst the underground workers than among the workers on the surface. The same peculiarity is well known to exist on the gold mines of the Witwatersrand, but the difference in the liability of the two classes of workers on these latter mines is less than at the De Beers Mines. This peculiarity is so constant and so well marked that but little acumen is required to arrive at the conclusion that the phenomenon is due to some factor, or factors, inherent in the conditions of underground employment. Many explanations for this greater liability have been suggested from time to time, *e.g.*, inefficient ventilation, the exhausting nature of the work, the irritation produced by inhaling dust and the fumes of explosives, the exposure to rapid variations of temperature, etc. The remedies hitherto proposed have been as various as the explanations offered, and have been equally speculative in character. It is not an exaggeration to say that volumes could be compiled of published literature of this class; the one essential which is lacking from all these efforts, however, is any evidence of an attempt having been made to submit the particular hypothesis to experimental proof. The very existence of the *vera causa* of the disease appears, moreover, to be almost ignored in all these misty speculations. One thing is certain, that Lobar Pneumonia is usually the result of invasion by the Pneumococcus; this being so, it seems to me more profitable to raise the resisting powers of the individual against this organism than to attempt to remove a large number of speculative environmental influences, some of which are practically ineradicable. These remarks should not be construed to imply that I advocate a disregard of proper hygienic standards, but that I consider that they should take a subordinate place to *specific* prophylactic procedures.

I visited the De Beers Mines during February, 1917, and investigated a number of cases of Pneumonia. The Pneumococcus of groups "A," "B" and "C" was found to be prevalent there, as on the gold mines. I also established the existence and identity of another group, which I designated "H"; during my short visit (less than one month) four cases with two deaths occurred which were due to infection by this group. This fact led to my using, on the De Beers Mines, a vaccine containing a representative of groups "A," "B," "C," and "H," with a total content of eight thousand millions of organisms per dose of 1 c.c.

The experimental period began on May the 1st, and ended on October the 31st, 1917. During these six months all new arrivals (totalling 6,466) were inoculated subcutaneously, on three occasions, and at seven days' intervals with the vaccine. The average length of service on these mines is only about five months, and by the end of the experimental period 797 of the 6,466 new arrivals who had been inoculated had already left the mines.

The experiment, unlike those on the Premier and Crown Mines, was initiated in a large *uninoculated* community. The uninoculated population numbered some 7,400 Natives, and these people, in

accordance with the principle of inoculating only the new arrivals, received no prophylactic injections. In consequence of this fact, and also on account of the relative shortness of the experimental period, the ratio of inoculated to uninoculated Natives did not become very large until towards the end of the experimental period. These considerations justify the conclusion that the maximum effects of the system of inoculation had not developed by the end of the experimental period; the returns recorded in Table IX. afford, however, very suggestive collateral evidence of its beneficial results. The returns in this table are given in a form different to that in previous tables, but the greater detail in which they appear affords, in the circumstances, a truer estimate of the results obtained.

Table IX. shows that in May (the month in which inoculation commenced) the incidence and mortality *amongst the uninoculated residents* increased very pronouncedly; this marked increase was, however, short-lived, and in the following month the number of deaths from Pneumonia was lower than in any preceding month of the year; this improvement was maintained throughout the remaining months of the experimental period.

When it is considered that Pneumonia was unusually prevalent among the uninoculated during the first month of the inoculation experiment, and bearing in mind the admittedly greater susceptibility of new arrivals to this disease, one may be permitted to hold the opinion that the results are, so far, very encouraging. These results are, moreover, not inconsistent with my previously expressed views concerning the benefit conferred on the uninoculated population by the systematic inoculation of all new arrivals. It can be predicted with some confidence that the returns for a completed inoculation year will show a lower death rate from Lobar Pneumonia on these mines than has ever been approached by them previously. Further investigations of the "residual deaths"* will disclose what group, or groups, of the Pneumococcus are responsible for them; it should then be possible to bring about a further and still more substantial reduction in the Pneumonia death-rate.

SECTION VI.

Discussion.

The extent to which the mortality from Lobar Pneumonia has been reduced on each of the three experimental mines, coincidently with systematic use of a specific group vaccine, has been shown in the preceding sections. The complete abolition of "A," "B" and "C" Pneumonia on the Crown Mines, following upon the immunization of the Native labour force with a vaccine containing a representative of these three groups only, removes the question of the efficacy of this

* The expression "residual deaths" is intended to signify those deaths from Lobar Pneumonia in which the infection was caused by any group of the Pneumococcus other than those contained in the vaccine.

TABLE IX.

The De Beers Diamond Mines, Pneumonia Returns for each Month from January to October, 1917, inclusive, together with the number of new arrivals (inoculated) during each month.

Month.	Average number of Natives employed, including new arrivals.	Number of new inoculated arrivals.	Number of Pneumonia cases.			Number of deaths from Pneumonia.		
			Inoculated.	Uninoculated.	Totals.	Inoculated.	Uninoculated.	Totals.
January ...	7,204	—	—	16	16	—	6	6
February ..	7,534	—	—	24	24	—	5	5
March ...	7,499	—	—	17	17	—	5	5
April	7,431	—	—	12	12	—	5	5
May*	7,456	1,102	4	34	38	1	13	14
June	7,462	971	2	12	14	1	3	4
July	7,381	954	9	9	18	1	1	2
August ...	7,381	1,072	5	7	12	1	2	3
September	7,423	1,112	4	3	7	1	0	1
October ...	7,541	1,255	6	3	9	2	0	2
Totals ...	—	6,466	30	137	167	7	40	47

* Experimental period began May 1st.

procedure from the realm of speculation to that of certainty. It is, however, by no means desirable that our efforts should end at this point. There are still a number of cases of and deaths from Pneumonia on the Crown Mines, in spite of the complete abolition of all those due to the Pneumococcus of groups "A," "B" and "C." It is very necessary to deal with these "residual" cases, and there is a likelihood, amounting almost to certainty, that this is both a possible and practicable undertaking. Inspection of Table VII. shows that during a period of nine months there were twelve deaths amongst the inoculated population on the Crown Mines, and that ten of these were due to the Pneumococcus belonging to one or other of the groups "E" (Pneumococcus mucosus), "H," "J" or "K." The way to further progress lies, therefore, in the inclusion in future vaccine of representatives of these groups in addition to those of "A," "B" and "C." An increase of the total number of cocci in each dose will, in these circumstances, probably be necessary; due care must be taken, however, that the strains employed are not so numerous as to prejudice the establishing of an adequate immunity against each.

When a more comprehensive vaccine, as suggested above, is in systematic use, a sustained investigation of the comparatively few fatal cases of Pneumonia that may still occur should rapidly lead to the identification of any further group of the Pneumococcus that has hitherto been obscured by the mass of material associated with the commoner groups.

Although no returns have been specially obtained to elucidate the possible effect of this inoculation upon the prevalence and characters of Pneumococcal Meningitis, and other forms of pneumococcal disease other than Lobar Pneumonia, beneficial results have most probably arisen. It is a significant fact that on the Crown Mines the death-rate from "Meningitis" has fallen to a point never previously approached.

Many people hold the opinion that Lobar Pneumonia predisposes to Pulmonary Tuberculosis, and the late Dr. G. A. Turner (Medical Officer of the Witwatersrand Native Labour Association) was a strong supporter of this hypothesis; I am personally unaware of any entirely reliable evidence on the point, but if the supposition be a correct one then the prevention of Pneumonia in mine Natives will have unexpectedly far-reaching effects.

This report deals with Pneumonia prophylaxis as it affects Natives; in Europeans, at all events in Johannesburg, I regard the problem as far simpler, inasmuch as Pneumonia in the latter is most commonly due to one group only, viz., "C"; it follows that a vaccine for their use need not contain so many strains, and consequently a smaller total number of organisms.

An investigation of the bacteriology of the Broncho-pneumonia which frequently, and the Lobar Pneumonia which occasionally, complicates an attack of Measles might prove of some advantage; for if it be found that these intercurrent diseases are usually due to

infections with specific pneumococcal groups, a timely immunization at the onset of the disease (Measles) might prevent a number of the fatalities in children.

I have not lost sight of the fact that a reduction of the mortality from Pneumonia has occurred of late amongst the whole population of Native mine labourers in the Transvaal. This is especially obvious during the present year, and critics have lost no time in expressing the opinion that this fact indicates that the reduction recorded on the experimental mines is merely part of a general decrease due to natural causes. This bare expression of opinion, however, constitutes a very glaring instance of *suppressio veri*, for the fact that, whilst there is a total of about 200,000 Native labourers, nearly three hundred thousand doses of pneumococcal vaccine have been issued by this Institute for their prophylactic inoculation during the past twenty-two months, is ignored. Until the statement that " A," " B " and " C " Pneumonia has been completely abolished under experimental conditions is refuted, all such speculative criticism is futile and scarcely deserves serious consideration.

There is no doubt that the ventilation of underground workings has been improved on many mines within recent years, and the dust-content of mine air has been greatly lessened, and these are hygienic improvements to which one might reasonably ascribe some of the recent decline in the Pneumonia mortality; but if this assumption is correct how can the high mortality which preceded inoculation on the Premier Diamond Mine be accounted for? This mine is, to all intents and purposes, a relatively shallow and open pit, about 300 feet in depth, half a mile in length, a quarter of a mile in breadth at the surface, and with a floor-area of seventy-eight acres; inadequate ventilation could hardly be accounted the responsible factor in this instance. Again, Tuberculosis, which should also respond to such improvements in mine hygiene, is unfortunately increasing rather than declining amongst the Native mine labourers.

Although the general death-rate from Pneumonia amongst all mine Natives has admittedly fallen during the last eighteen months it still remains, nevertheless, considerably greater than that now prevailing amongst the Native populations of the three experimental mines; and in making such a comparison it must be remembered that the relative Pneumonia mortality for these three mines, prior to inoculation, consistently exceeded the rate for all the Native labourers of the Transvaal.

Without attempting to discuss in detail all the circumstances associated with this general decline in Pneumonia mortality, I desire to draw attention to the following facts: The Pneumonia death-rate officially published every month by the Native Labour Department of the Government refers to all Native labourers employed on mines and works in the proclaimed labour districts of the Transvaal; it therefore includes the Crown and Premier Mines, where the Pneumonia mortality has greatly decreased consequently upon prophylactic

inoculation. On several other Transvaal mines—having a total complement of some fifty thousand Natives—inoculation of all new arrivals has been systematically practised for as long a period, in some cases, as two years. And lastly, the number of Natives employed on works other than mines has greatly increased during the last two years; such Natives suffer from Pneumonia to an insignificant extent as compared with the mine labourers.

I do not wish to suggest that the whole of the recent improvement in the mortality from Pneumonia can be accounted for by these circumstances, but collectively they must have had a considerable effect, and the criticisms referred to above must carry the stigma of prejudice so long as important considerations of this nature are studiously avoided.

The fact that the recent decline in the Pneumonia death-rate has been general is the only line of attack left to those who cannot see any hopeful prospect in Pneumonia prophylaxis. The argument is, in my opinion, a weak one, since it ignores evidence upon which absolute reliance can be placed, viz., that the prevalent group-types of Pneumonia have been abolished by the inoculation of a vaccine comprising homologous pneumococcal groups.

The future will most assuredly prove that when inoculation is carried out with a vaccine containing certain additional groups of the Pneumococcus, Lobar Pneumonia will become an almost negligible factor in the general mortality from disease.

SECTION VII.

Conclusions.

(1) That from 63 to 77 per cent. of all cases of Lobar Pneumonia occurring under normal conditions in Native mine labourers in the Transvaal are caused by the Pneumococcus of one or other of the groups "A," "B" or "C."

(2) That Native mine labourers who have been suitably inoculated with a vaccine comprising certain pneumococcal groups are immune from Lobar Pneumonia due to such groups.

(3) That three subcutaneous inoculations of one c.c. of a vaccine containing representatives of groups "A," "B" and "C," and having a total content of seven thousand millions of cocci per c.c., have rendered a large Native mine population (Crown Mines) absolutely resistant to pneumonic infection by any of these groups during the observed experimental period of nine months.

(4) That during the experimental period, when Pneumonia due to groups "A," "B" and "C" had been completely abolished on the Crown Mines, these three groups were still prevalent (in about their normal ratio to all groups) amongst the pneumonic Natives on those mines where inoculation was not practised.

(5) That the unequivocal character of the results obtained on the Crown Mines suggests that triple inoculation with a three-group vaccine in doses of *less* than seven thousand millions of cocci would probably afford an efficient immunity against these three groups.

(6) That the Pneumococcus of groups "E," "H," "J," and "K" are probably of sufficient epidemiological importance to warrant their being included in future vaccine intended for Native mine labourers.

(7) That the alteration of relative group prevalence by means of specific group inoculation is a more critical test of the efficacy of Pneumonia prophylaxis than the simultaneous comparison of Pneumonia rates in inoculated and uninoculated (control) groups—when such comparison is based upon the erroneous assumption that all cases of the disease due to the Pneumococcus are bacteriologically indistinguishable.

I am indebted to a number of individuals for their helpful assistance—to Dr. W. Watkins-Pitchford, Director of this Institute, for much time and trouble spent in the official organisation of these experiments; to Dr. H. A. Loeser, Chief Medical Officer, Crown Mines, Dr. A. J. McClymont, Medical Officer, Premier Diamond Mine, and Drs. A. Fuller and J. E. Mackenzie, Medical Officers of De Beers Diamond Mines, for the careful superintendence of the inoculations carried out on their respective mines. I am especially indebted to Miss Alice G. Lambert for very valuable assistance in carrying out the series of tests in connection with the experiments on the Crown Mines, De Beers Mines and others—an assistance which has been characterized throughout by accuracy, conscientiousness, and enthusiasm. Mr. J. R. Cooke has helped to make the preparing of this vaccine on a large scale a practical undertaking, and has invariably confronted the many disappointments incidental to the work with a most cheerful resourcefulness. Finally, I have to acknowledge a special debt of gratitude to Mr. E. A. Wallers, President of the Chamber of Mines, who, since the inception of my work on this subject in 1913, has encouraged me by granting facilities in many directions; whatever merit these investigations may now possess is largely due to his practical sympathy in the early stages.

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A SURVEY OF THE INTESTINAL ENTOZOA, BOTH PROTOZOAL AND HELMINTHIC, OBSERVED AMONG NATIVES IN JOHANNESBURG FROM JUNE TO NOVEMBER, 1917.

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WITH TWO PLATES.

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A SURVEY OF THE INTESTINAL ENTOZOA, BOTH PROTOZOAL AND HELMINTHIC, OBSERVED AMONG NATIVES IN JOHANNESBURG, FROM JUNE TO NOVEMBER, 1917.

I.—INTRODUCTION.

In a tropical or sub-tropical country, where there is a large resident, yet fluctuating, native population, as well as a European community, it is desirable to have definite information as to what intestinal animal parasites are present among the coloured races, and the possibilities of sporadic or epidemic diseases of entozoal origin that may arise among these people.

During the six months June to November, 1917, I was engaged in a systematic examination of the stools of male Natives in hospital in Johannesburg, whether for surgical or medical reasons, suffering chiefly from other than intestinal complaints, while the stools of a smaller number of similarly circumstanced white men were examined for Entozoa. A small number of dysenteric stools were also examined, which may be considered as representative of those cases of dysentery which normally occur among the indigenous population under non-war conditions. A detailed examination of the protozoal and helminthic contents of the intestines of over 100 Natives examined *post mortem* was also made. The work enables a preliminary statement to be made as to the nature and distribution of the animal parasites among the Natives. It further sheds some light on the existence of a very limited protozoal fauna that may, perhaps, be considered normal to the human intestine, although exceedingly limited in extent as compared with the abundant bacterial flora that is usually present. Definite information as to the action and effects of certain parasitic Protozoa upon the human intestine has also been obtained.

The results, while not indicating the presence of parasites causing diseases of marked economic importance, yet clearly indicate that certain Protozoa do occur, *e.g.*, *Giardia* (*Lamblia*) *intestinalis*, which, under circumstances causing general lowered vitality among many Natives, would multiply rapidly, and produce intestinal diseases, such as diarrhœa, in epidemic form. Several comments regarding such Entozoa will be found in the paper.

While careful work was done by the late Dr. G. A. Turner (1907, 1910) in connection with the helminthic parasites occurring among mine natives, the present paper deals with both protozoal and helminthic parasites, not only of mine boys but also of the inhabitants of the city as represented in the native wards of the Johannesburg Hospital. It is believed to be the first detailed account of the parasitic Protozoa observed in the intestines and stools of Natives working on the Witwatersrand.

II.—MATERIAL AND METHODS.

As before-mentioned, the stools examined were those of male patients in the native wards of the General Hospital, Johannesburg; cases of amœbic dysentery form only a small part of the investigation, though such stools were examined microscopically and in some cases every day throughout the illness. At a minimum, three examinations were made of the stools of each patient at intervals during his stay in hospital, and the total protozoal and helminthic content was thus estimated in each case. The history of the patient was ascertained, and the details noted.

In each case, a stool passed soon after the patient was admitted to hospital was examined. In accordance with the hospital routine, an aperient, such as magnesium sulphate, was given to each patient on admission; the first stool was thus practically fluid, and its entozoal content comprised representatives of the great majority of the animal parasites present in the intestinal canal. Subsequent examinations sometimes revealed the presence of additional entozoa, but after a number of cases in which daily examinations were made for twenty days, the conclusion was reached that in the majority of cases the additional examinations yielded no more parasites than the first three had done.

The method of examination was as follows:—A bedpan from each patient was kept by the ward orderly, labelled with the name of the ward and bed number of the patient, and deposited in a small room provided with water, lavatory sinks, etc. In the morning I went to this room and examined the stools thus saved. In each case, a small bottle or swab was used, and a typical sample of the stool placed therein, special care being taken to include mucus or blood, if present, tapeworm segments and any other obvious helminthic parasites. A note was made as to the general nature of the stool. The average sample of stool thus secured was about 10 c.c. in volume. The remainder of the stool was then broken up and sedimented in order to detect worms, if present. Finally, the history of the patient was obtained from his bed-letter. The detailed examination of the fæces was made in the laboratory at the adjoining S.A. Institute for Medical Research. Fresh preparations were always employed, and were examined in the ordinary way, while in some cases the paraboloid condenser was also used. Stained preparations were made in special cases.

Concentration methods for the detection of protozoal cysts and helminthic ova were also tried, but it was found that such were of relatively little service, and, as they took too long for practical purposes, they were abandoned after a time.

It will, perhaps, be convenient to consider first the parasites found in stools, and then those detected at post-mortem examinations.

I would wish here to return hearty thanks to Dr. Watkins-Pitchford, Director of the Institute, and to the Superintendent of the Johannesburg Hospital and his staff, particularly to Sisters Cassidy and O'Rafferty, for the cordial co-operation which has greatly facilitated my work.

The post-mortem examinations of intestines were made at the laboratory of the S.A. Institute for Medical Research; the deceased subjects were "mine boys," or very occasionally new recruits, who had died at the Hospital of the Witwatersrand Native Labour Association.

Scrapings of the intestinal mucosa were examined from pylorus to anus, as well as preparations of the intestinal contents. The history of each case examined was also obtained. I would thank most heartily Dr. A. I. Girdwood and Dr. A. Percival Watkins, of the W.N.L.A. Hospital, for their invariable kindness and courtesy to me in connection with this work.

SECTION A.—ENTOZOA FOUND IN STOOLS.

III.—DISTRIBUTION OF FÆCAL ENTOZOA AMONGST THE VARIOUS TRIBES EXAMINED.

As a result of detailed examination of the stools of Natives of the various races, tribes or divisions of tribes, set forth in Table I overleaf, it was found that 56·8 per cent. of those examined contained parasitic Protozoa, or Helminthes, or both. Single infections, that is, infections with one species of organism only, occurred, both Protista and Helminthes being represented. Double infections consisted either of two kinds of Protist or of one Protist and one Helminth, double helminthic infections not being found in stools of hospital patients, though a few instances were observed *post mortem*. The multiple infections consisted of three or more Protists, or of two or more Protists and one Helminth found in the same stool.

In the adjoined table, the number of infected patients examined of each race, tribe, or division of tribe is indicated, together with the number actually found to be infected, and the character of the infection (whether single Protist, single Helminth, double or multiple entozoal infections).

The term "Protist" is used throughout to comprise parasitic Protozoa in the restricted sense, together with Spirochætacea and Blastocystis.

TABLE I.

Tribal Distribution of Patients, with General Character of Entozoal Infections of Stools.

Race or Tribe.	Number Examined.	Number Infected.	Number infected with Protista only.	Number infected with Helminthes only.	Number infected with both Protista and Helminthes.	Single Protistan Infections.	Single Helminthic Infections.	Double Entozoal Infections.	Multiple Entozoal Infections.
Cape	61	33	31	1	1	15	1	15	2
Transkei—									
M'Xosa	17	9	9	0	0	4	0	5	0
Pondo	4	4	3	0	1	2	0	0	2
Tambooki	1	1	1	0	0	0	0	1	0
Fingo	1	1	1	0	0	0	0	1	0
Baca	1	1	0	0	1	0	0	0	1
Kaffir	1	0	0	0	0	0	0	0	0
Hottentot	9	5	4	0	1	1	0	1	3
Griqua	1	1	1	0	0	0	0	1	0
Basutos—									
Basuto	27	17	14	2	1	9	2	5	1
M'Kalonga	1	1	0	0	1	0	0	1	0
M'Naring	1	0	0	0	0	0	0	0	0
M'Gato	1	1	1	0	0	1	0	0	0
M'Nvali	1	0	0	0	0	0	0	0	0
Marolo	4	3	3	0	0	1	0	2	0
Bechuana	3	1	1	0	0	1	0	0	0
Baralong	2	2	2	0	0	1	0	1	0

TABLE I.—(*Continued*).

Race or Tribe.	Number Examined.	Number Infected.	Number infected with Protista only.	Number infected with Helminthes only.	Number infected with both Protista and Helminthes.	Single Protistan Infections.	Single Helminthic Infections.	Double Entozoal Infections.	Multiple Entozoal Infections.
Zulu	97	56	32	10	14	20	10	16	10
Swazi and Dwazi	6	4	4	0	0	2	0	0	2
Msutu	64	29	28	1	0	18	1	8	2
East Coast—									
Delagoa Bay	1	0	0	0	0	0	0	0	0
Tonga	1	1	1	0	0	0	0	1	0
Shangaan	24	17	13	2	2	7	2	7	1
Portuguese East African	5	2	2	0	0	1	0	0	1
Moçambique	6	5	4	1	0	3	1	1	0
Blantyre	3	1	0	0	1	0	0	1	0
Nyambaan	4	2	2	0	0	2	0	0	0
M'Chopi	2	1	1	0	0	0	0	1	0
Bantu (Uganda)	1	1	0	1	0	0	1	0	0
Nyasa	1	1	1	0	0	1	0	0	0
Somali	1	0	0	0	0	0	0	0	0
Indian	20	12	10	1	1	3	1	6	2
Chinese	3	1	1	0	0	1	0	0	0
TOTALS	375	213	170	19	24	93	19	74*	27

*Of these 74 cases, 60 were double Protist infections and the remaining 14 were mixed double infections.

From Table I it will be seen that one or more parasites were harboured by a relatively large proportion of the Natives, the Protista being far more numerous than the Helminthes. The largest number of Natives examined belonged to the Zulu race, while Msutus, Cape coloured persons, Basutos and Shangaans comprised the bulk of the remainder. The value of observations in regard to percentage infection in the majority of the remaining cases is almost nullified by the small numbers of individuals involved, though they were all that were available. Of the tribes sufficiently represented to yield a fairly accurate estimate, it was found that approximately 70·8 per cent. of the Shangaans, 52·57 per cent. of the Zulus, 64·7 per cent. of the Basutos (with their sub-families), 45·3 per cent. of the Msutus and 54 per cent. of the Cape coloured people harboured parasites. It may also be noted that the helminthic infections were in the main confined to these tribes.

IV.—SINGLE PROTIST INFECTIONS OF STOOLS, WITH THEIR TRIBAL DISTRIBUTION.

Infections with a single Protist were found in the stools of 93 Natives. The number of subjects in whom the Protists occurred were as follows: —

TABLE II.

Single Protist Infections of Stools, with Numbers of Cases in which each Infection was found.

Entamoeba coli	42
Giardia (Lamblia) intestinalis	3
Trichomonas intestinalis	2
Chilomastix mesnili	1
Prowazekia cruzi	3
Cercomonas parva	5
Spirochæta eurygyrata	29
"Free-living" Amoebae	2
Blastocystis hominis	6
	93

The examinations of the single infections show that *Entamoeba coli* and *Spirochaeta eurygyrata* occurred most frequently in these stools. It was noted that *S. eurygyrata* was more abundant in stools of patients who had temporary intestinal troubles consequent mainly on a change of diet.

In six cases, parasitic Flagellata, namely, *Giardia intestinalis*, *Trichomonas intestinalis*, and *Chilomastix mesnili*, which are often associated with flagellate diarrhœa, were present; vague accounts of intestinal trouble were given by each of the patients concerned, and they were placed under appropriate treatment.

The tribal distribution of the single protistan infections is shown in the following table: —

TABLE III.

Tribal Distribution of Patients with Single Protist Infections of Stools.

Tribe.	Entamœba coli.	Giardia intestinalis.	Chilomastix mesnili.	Trichomonas intestinalis.	Spirochæta eurygyrata.	Other Protozoa.	Blastocystis hominis
Cape	6	—	—	—	4	2 Cercomonas 1 Prowazekia	2
M'Xosa	—	—	—	1	3	—	—
Pondo	1	—	—	—	1	—	—
Hottentot	—	1	—	—	—	—	—
Basuto	6	—	1	—	1	—	1
M'Gato	1	—	—	—	—	—	—
Marolo	1	—	—	—	—	—	—
Bechuana	1	—	—	—	—	—	—
Baralong	—	1	—	—	—	—	—
Zulu	9	—	—	1	6	2 Cercomonas 1 Prowazekia	1
Swazi and Dwazi	1	—	—	—	1	—	—
Msutu	9	—	—	—	6	1 "Free-living" Amoeba	2
Shangaan	3	—	—	—	4	—	—
Portuguese East African	1	—	—	—	—	—	—
Moçambique	1	—	—	—	—	1 Prowazekia 1 "Free-living" Amoeba	—
Nyambaan	—	1	—	—	1	—	—
Nyasa	1	—	—	—	—	—	—
Indian	1	—	—	—	1	1 Cercomonas	—
Chinese	—	—	—	—	1	—	—
TOTALS	42	3	1	2	29	5 Cercomonas 3 Prowazekia 2 "Free-living" Amoeba	6

It is evident that *Entamœba coli* and *Spirochæta eurygyrata* were the most widely distributed Protists among the tribes, the parasitic Flagellata being present in relatively few cases. However, organisms which, when present in numbers, produce flagellate diarrhœas occurred in six patients representing six different tribes. As the Natives concerned came from Cape Colony, the Transkei, Zululand, Basutoland, Bechuanaland and the East Coast, it may be inferred that the geographical distribution of Giardia, Chilomastix, and Trichomonas is a wide one in the sub-continent, and it is probable that a more extended series of examinations would show a wider tribal distribution of these organisms.

V.—SINGLE HELMINTHIC INFECTIONS OF STOOLS.

Helminthic parasites, unaccompanied by Protista, were observed in the dejecta of 19 patients, in the specimens from each of whom one kind of worm only was found. Both Cestoda and Nematoda were represented. Among the Cestoda, two species of Tænia and one of Hymenolepis were found. *Tænia solium* occurred in one Zulu, two Basutos, one Moçambique, and one Indian; *Tænia saginata* was found in the fæces of eight Zulus, one Basuto, and one Shangaan; while *Hymenolepis nana* was parasitic in one Cape coloured man.

The Nematoda observed were *Oxyuris vermicularis* in a Shangaan, and *Ascaris lumbricoides* in a Msutu and a Zulu.

Details regarding Helminthes associated in the same patient with Protista will be given subsequently. A summary of the single helminthic infections is given in Table IV.

TABLE IV.

Single Helminthic Infections of Stools, with Numbers of Cases in which each Infection was found.

Tænia solium	5
Tænia saginata	10
Hymenolepis nana	1
Oxyuris vermicularis	1
Ascaris lumbricoides	2
	19

VI.—DOUBLE PROTIST INFECTIONS OF STOOLS.

In each of the 60 cases now to be considered, two Protists co-existed in the same patient. From a series of observations, it was found that one of the parasites was at times much more abundant than the other, and also that on some occasions one might be absent from the stool while the other was abundant, although both had previously co-existed. In other words, both here and in the multiple infections

to be discussed later, there was a periodicity in the appearance of the maximum numbers of the different parasites, just as was determined for the various Protista observed in European patients by Fantham (1916).

The different combinations of Protists, and the numbers of instances of each combination observed, are set forth in Table V.

TABLE V.

Double Protist Infections of Stools, with Numbers of Cases in which each Infection was found.

			No.
Entamœba coli	and	"*Free-living*" *Amœbæ*	1
,, ,,	,,	*Giardia intestinalis*	2
,, ,,	,,	*Trichomonas intestinalis*	3
,, ,,	,,	*Chilomastix mesnili*	1
,, ,,	,,	*Prowazekia cruzi*	1
,, ,,	,,	*Cercomonas parva*	1
,, ,,	,,	*Bodo stercoralis*	1
,, ,,	,,	*Spirochæta eurygyrata*	38
,, ,,	,,	*Blastocystis hominis*	6
Giardia intestinalis	and	*Trichomonas hominis*	1
,, ,,	,,	*Spirochæta eurygyrata*	1
Spirochæta eurygyrata	and	"*Free-living*" *Amœbæ*	1
,, ,,	,,	*Blastocystis hominis*	1
Blastocystis hominis	and	"*Free-living*" *Amœbæ*	2

The grouping of the tribes with respect to the Protista found as double infections is given in Table VI.

TABLE VI.

Tribal Distribution of Patients with Double Protist Infections of Stools.

Tribe.	Double Protist Infections			No.
Cape	*Entamœba coli*	and	"*Free-living*" *Amœbæ*	1
	Entamœba coli	,,	*Chilomastix mesnili*	1
	Entamœba coli	,,	*Trichomonas intestinalis*	1
	Entamœba coli	,,	*Spirochæta eurygyrata*	9
	Entamœba coli	,,	*Blastocystis hominis*	1
	Giardia intestinalis	,,	*Trichomonas intestinalis*	1

TABLE VI.—(*Continued.*)

Tribe.	Double Protist Infections.			No.
M'Xosa	*Entamœba coli*	and	*Cercomonas parva*	1
	Entamœba coli	,,	*Spirochæta eurygyrata*	4
Tambooki	"*Free-living*" *Amœbæ*	,,	*Blastocystis hominis*	1
Fingo	*Entamœba coli*	,,	*Giardia intestinalis*	1
Hottentot	*Entamœba coli*	,,	*Blastocystis hominis*	1
Griqua	*Entamœba coli*	,,	*Spirochæta eurygyrata*	1
Basuto	*Entamœba coli*	,,	*Bodo stercoralis*	1
	Entamœba coli	,,	*Spirochæta eurygyrata*	1
	Entamœba coli	,,	*Blastocystis hominis*	1
	"*Free-living*" *Amœbæ*	,,	*Spirochæta eurygyrata*	1
Marolo	*Entamœba coli*	,,	*Spirochæta eurygyrata*	1
	"*Free-living*" *Amœbæ*	,,	*Blastocystis hominis*	1
Baralong	*Entamœba coli*	,,	*Spirochæta eurygyrata*	1
Zulu	*Entamœba coli*	,,	*Trichomonas intestinalis*	1
	Entamœba coli	,,	*Spirochæta eurygyrata*	5
	Entamœba coli	,,	*Blastocystis hominis*	1
	Giardia intestinalis	,,	*Spirochæta eurygyrata*	1
Msutu	*Entamœba coli*	,,	*Giardia intestinalis*	1
	Entamœba coli	,,	*Spirochæta eurygyrata*	5
	Entamœba coli	,,	*Blastocystis hominis*	2
Tonga	*Entamœba coli*	,,	*Spirochæta eurygyrata*	1
Shangaan	*Entamœba coli*	,,	*Prowazekia cruzi*	1
	Entamœba coli	,,	*Spirochæta eurygyrata*	5
Moçambique	*Entamœba coli*	,,	*Spirochæta eurygyrata*	1
M'Chopi	*Entamœba coli*	,,	*Spirochæta eurygyrata*	1
Indian	*Entamœba coli*	,,	*Trichomonas intestinalis*	1
	Entamœba coli	,,	*Spirochæta eurygyrata*	3
	Spirochæta eurygyrata	,,	*Blastocystis hominis*	1

It may be noted that among these double Protist infections, *Giardia intestinalis*, *Chilomastix mesnili*, and *Trichomonas intestinalis* occurred in several cases, while one double infection of Giardia and Trichomonas was observed. Here, as with the single infections of the

afore-mentioned parasites, there was a vague history of intestinal trouble, and the man who harboured both Giardia and Trichomonas definitely suffered from alternating periods of constipation and diarrhœa, as was observed by Fantham and myself in the case of European military patients suffering from the same infections acquired in Gallipoli and Salonika. The most frequent double infection was that of *Entamœba coli* and *Spirochæta curygyrata*.

VII.—MIXED DOUBLE INFECTIONS OF STOOLS WITH PROTISTA AND HELMINTHES.

In fourteen cases it was found that the stools of the patients contained both Helminthes and Protista. A simultaneous infection of the stools of a hospital patient with two different Helminthes was not observed, although two and three different genera of worms were obtained, as will be shown later, from the intestines of "mine-boys" examined in detail *post mortem*. Both Cestode and Trematode parasites were found in the stools of the patients, the ova usually being first detected and the worms themselves being subsequently recovered after appropriate anti-helminthic treatment.

The mixed double infections grouped according to the parasites present are given in Table VII.

TABLE VII.

Mixed Double Entozoal Infections of Stools, with Numbers of Cases in which each Infection was found.

			No.
Cestoda and Protista	*Tænia solium*	and *Entamœba coli*	1
	Tænia solium	,, *Spirochæta curygyrata*	1
	Tænia solium	,, *Blastocystis hominis*	1
	Tænia saginata	,, *Entamœba coli*	7
	Tænia saginata	,, *Giardia intestinalis*	1
	Tænia saginata	,, *Spirochæta curygyrata*	1
Trematoda and Protista	*Schistosoma (Bilharzia) mansoni*	,, *Cercomonas parva*	1
	Schistosoma (Bilharzia) mansoni	,, *Entamœba coli*	1

The tribal distribution of the mixed double entozoal infections in stools is shown in Table VIII.

TABLE VIII.

Tribal Distribution of Patients with Mixed Double Entozoal Infections of Stools.

Tribe.	Mixed Double Entozoal Infection.			No.
Zulu	*Tænia solium*	and	*Entamœba coli*	1
	Tænia solium	,,	*Spirochæta eurygyrata*	1
	Tænia saginata	,,	*Entamœba coli*	4
	Tænia saginata	,,	*Spirochæta eurygyrata*	1
	Schistosoma mansoni	,,	*Entamœba coli*	1
Cape	*Tænia saginata*	,,	*Entamœba coli*	1
Basuto	*Tænia saginata*	,,	*Entamœba coli*	1
M'Kalonga	*Tænia saginata*	,,	*Giardia intestinalis*	1
Shangaan	*Tænia solium*	,,	*Blastocystis hominis*	1
Blantyre	*Schistosoma mansoni*	,,	*Cercomonas parva*	1
Indian	*Tænia saginata*	,,	*Entamœba coli*	1

VIII.—MULTIPLE PROTIST INFECTIONS OF STOOLS.

The stools of 27 of the men examined contained three or more parasites. As in the double infections, there were pure protistan infections (17 cases) and mixed infections of Protista and Helminthes (10 cases). In 16 cases, three Protists were present, while in the 17th case four Protists occurred simultaneously. A periodicity in the time of appearance of the different organisms in maximum numbers in the stools was observed.

The multiple Protist infections, grouped according to the parasites present, are indicated in Table IX, together with the number of cases in which each infection occurred.

TABLE IX.

Multiple Protist Infections of Stools.

				No.
Entamœba coli,	*Giardia intestinalis*	and	*Spirochæta eurygyrata*	4
,, ,,	*Giardia intestinalis*	,,	"*Free-living*" *Amœbæ*	1
,, ,,	*Chilomastix mesnili*	,,	*Spirochæta eurygyrata*	4
,, ,,	*Spirochæta eurygyrata*	,,	"*Free-living*" *Amœbæ*	1
,, ,,	*Spirochæta eurygyrata*	,,	*Blastocystis hominis*	4
,, ,,	*Cercomonas parva*	,,	*Spirochæta eurygyrata*	1
,, ,,	*Cercomonas parva*	,,	*Isospora bigemina* var. *hominis*	1
Entamœba coli, *Giardia intestinalis*,	*Spirochæta eurygyrata*	and	*Blastocystis hominis*	1

The tribal distribution of the multiple Protists in stools was as follows:—

TABLE X.

Tribal Distribution of Patients with Multiple Protist Infections of Stools.

Tribe.	Multiple Protist Infections.		No.
Cape	*Entamœba coli*, "*Free-living*" *Amœbæ*	and *Spirochæta curygyrata*	1
	Entamœba coli, *Spirochæta curygyrata*	and *Blastocystis hominis*	1
Pondo	*Entamœba coli*, *Giardia intestinalis*, *Spirochæta curygyrata*	and *Blastocystis hominis*	1
Hottentot	*Entamœba coli*, *Giardia intestinalis*	and *Spirochæta curygyrata*	1
	Entamœba coli, *Cercomonas parva*	and *Isospora bigemina* var. *hominis*	1
Basuto	*Entamœba coli*, *Giardia intestinalis*	and "*Free-living*" *Amœbæ*	1
Swazi	*Entamœba coli*, *Giardia intestinalis*	and *Spirochæta curygyrata*	1
	Entamœba coli, *Chilomastix mesnili*	and *Spirochæta curygyrata*	1
Zulu	*Entamœba coli*, *Chilomastix mesnili*	and *Spirochæta curygyrata*	1
	Entamœba coli, *Cercomonas parva*	and *Spirochæta curygyrata*	1
	Entamœba coli, *Spirochæta curygyrata*	and *Blastocystis hominis*	2
Msutu	*Entamœba coli*, *Chilomastix mesnili*	and *Spirochæta curygyrata*	1
	Entamœba coli, *Spirochæta curygyrata*	and *Blastocystis hominis*	1
Portuguese East African	*Entamœba coli*, *Chilomastix mesnili*	and *Spirochæta curygyrata*	1
Indian	*Entamœba coli*, *Giardia intestinalis*	and *Spirochæta curygyrata*	2

It may be noted that ten out of the seventeen infections listed in Table X were with parasitic Flagellata (*Giardia intestinalis*, *Chilomastix mesnili*) which are capable of pathogenic effects, when abnormal conditions lead to their multiplicative activity, or when they later reach a susceptible subject.

IX.—MIXED MULTIPLE INFECTIONS OBSERVED IN STOOLS.

The 10 multiple infections that comprised both Protist and Helminth parasites are shown in Table XI, together with the number of cases in which they were observed.

TABLE XI.

Mixed Multiple Infections in Stools.

			No.
Tænia solium,	*Entamœba coli*	and *Spirochæta eurygyrata*	1
Tænia solium,	*Cercomonas parva*	and *Blastocystis hominis*	1
Tænia solium,	*Spirochæta eurygyrata*	and *Blastocystis hominis*	1
Tænia saginata,	*Entamœba coli*	and *Giardia intestinalis*	1
Tænia saginata,	*Entamœba coli*	and *Bodo stercoralis*	1
Tænia saginata,	*Entamœba coli*	and *Spirochæta eurygyrata*	3
Tænia saginata,	*Entamœba coli*	and *Blastocystis hominis*	1
Tænia saginata,	*Trichomonas hominis*	and *Spirochæta eurygyrata*	1

Bodo stercoralis, already listed in Tables V and VI (pp. 11, 12), is a Flagellate from human fæces believed to be new to science and to be recorded in this paper for the first time. Details regarding its structure are given on pp. 43, 44, and it is figured on Plate I, Fig. 19.

The tribal distribution of the mixed multiple infections is given in Table XII.

TABLE XII.

Tribal Distribution of Patients with Mixed Multiple Infections of Stools.

Tribe.	Combination of Parasites.			No.
Pondo	*Tænia saginata*,	*Entamœba coli*	and *Blastocystis hominis*	1
Baca	*Tænia saginata*,	*Entamœba coli*	and *Spirochæta curygyrata*	1
Hottentot	*Tænia saginata*,	*Entamœba coli*	and *Giardia intestinalis*	1
Zulu	*Tænia solium*,	*Entamœba coli*	and *Spirochæta curygyrata*	1
	Tænia solium,	*Cercomonas parva*	and *Blastocystis hominis*	1
	Tænia saginata,	*Entamœba coli*	and *Bodo stercoralis*	1
	Tænia saginata,	*Entamœba coli*	and *Spirochæta curygyrata*	2
	Tænia saginata,	*Trichomonas hominis*	and *Spirochæta curygyrata*	1
Shangaan	*Tænia solium*,	*Spirochæta curygyrata*	and *Blastocystis hominis*	1

It is of interest to note that in almost all cases of infection with tapeworms, whether single, double or multiple infections, the patients were men of classes mostly in close contact with white races—cooks, kitchen boys, house boys, and store boys furnished most of the cases, while farm-hands were also parasitised. Although the normal hosts of the bladderworm stages are domestic animals, for example, the ox and the pig, such stages are sometimes known in man. Hence the possible communication of Cestode infections to the white population needs to be borne in mind when such intimate association of infected natives with the food of Europeans, and sometimes with European children, is in vogue.

X.—GENERAL SURVEY OF THE PROTIST PARASITES OBSERVED IN THE STOOLS OF NATIVES.

It may be advisable at this stage to show the number of times each organism listed was actually recorded, and with this object in view, one table showing the total number of times each Protist was recorded in the various tribesmen, and a second one giving similar particulars for the Helminth infections, have been prepared (see Tables XIII, XIV) from the preceding data.

As a general statement, the Protist parasites most commonly occurring were *Entamœba coli* and *Spirochæta eurygyrata*. While both organisms are non-pathogenic in the general opinion, it has been noted by several workers that *E. coli* multiplies more vigorously in an unhealthy intestine than in a healthy one, while Fantham (1916) has found that *S. eurygyrata* is much more numerous in diarrhœic or dysenteric stools than in normal ones; an observation I can readily confirm. The presence of either *E. coli* or *S. eurygyrata* in very large numbers, then, may serve as an early indication of latent or potential intestinal disorders.

The Mastigophora associated with flagellate diarrhœas were observed on relatively few occasions. Nevertheless, the number of tribes represented is fairly considerable—Cape, M'Xosa, Basuto, Zulu, Msutu, Portuguese East African and Indians being infected. A wide geographical distribution of these potentially pathogenic flagellates is also indicated.

In connection with flagellate diarrhœa, I may state that I was able, during the course of these observations, to transmit Giardia, Trichomonas and Chilomastix of human origin to clean white rats by allowing their food to be contaminated with the excrement of cockroaches (*Periplaneta orientalis* and *P. americana*) that had been given access to infected stools and had fed thereon. The rôle of cockroaches as transmitters of flagellate diarrhœas of man can be readily understood, especially in semi-tropical and tropical countries, where their access to fæcal matter is relatively easy.

Another occurrence of scientific interest was the finding of *Isospora bigemina* var. *hominis* in the stools of a Hottentot. (See Table X). This parasite has previously been reported with certainty only in the stools of a few Europeans in Europe, who had contracted the infection while on active service in the Mediterranean War Zone.

The organism is evidently not restricted to Europeans, as the fairly heavy infection of the Hottentot showed. It may be mentioned that I have found the parasite also in the excrement of a South African of Dutch descent, who had lived all his life in South Africa, mainly in the Transvaal. His condition at first was thought to be amœbic dysentery, but *Isospora bigemina* var. *hominis* was the only Protozoön present (see p. 27), and with its destruction and elimination the symptoms also disappeared. By animal experiments (Fantham, 1917) it has been shown that this Isospora has a definite pathogenic action, and the possible presence of the organism should be borne in mind in cases of unusual dysenteric illnesses.

Cercomonas parva was observed at ten examinations. This organism has also been observed by Fantham (1916) and myself (1916) in military patients from Gallipoli, in whom no other parasitic agent of disease could be found. While its pathogenicity has not been fully tested by animal experiments, it may be regarded as a suspect. *Prowazekia cruzi*, now reported in African natives, has previously been found in cases of diarrhœa in South America.

Blastocystis hominis, an organism usually considered to be of fungoid origin, but possibly a stage of a Flagellate, has been frequently observed in the stools of military patients from the European War Zone, and its geographical distribution is now extended to South and East Africa.

The organisms now described as "Free-living" Amœbæ—of the *limax* type—can hardly be regarded as normal inhabitants of the human intestine. They were usually present in the encysted condition and resembled, in structure, the cysts of amœbæ that occur on vegetable matter, in moss, and on damp earth. It is probable that they had been ingested with vegetable food—similar amœbæ were obtained by me from lettuce leaves grown near Johannesburg on one occasion—and had passed through the human body, finally leaving it in an encysted condition. The "free-living" Amœbæ that I observed were not the same as those recently described under the name of *Entamœba nana*, which organism was not found.

It may be mentioned that, as a result of these systematic stool examinations, several carriers of amœbic dysentery were detected, the men being detained in hospital and treated until *Entamœba histolytica* was absent from their stools. Such carrier cases are not included in the tables already presented, but their detection gives an increased value to systematic stool examinations.

As will be seen from Table XIII, the total number of times Protista were recorded from the stools of Natives during the investigation was 299. However, the number of individuals harbouring the Protista was only 194, as will be gathered from Table I, columns 3 and 5, or from Tables II, V, VII, IX, XI.

TABLE XIII.

Number of Times each Protist was Recorded in Stools of Natives.

Tribe.	Entamœba coli.	Giardia intestinalis.	Chilomastix mesnili.	Trichomonas intestinalis.	Spirochæta eurygyrata	Prowazekia cruzi.	Bodo stercoralis.	Cercomonas parva.	Isospora bigemina var. hominis.	"Free-living" Amœbæ.	Blastocystis hominis.
Cape	22	1	1	2	15	1	—	2	—	2	4
Transkei—											
M'Xosa ...	5	—	—	1	7	—	—	1	—	—	—
Pondo... ...	3	1	—	—	2	—	—	—	—	—	2
Tambooki ...	—	—	—	—	—	—	—	—	—	1	1
Fingo	1	1	—	—	—	—	—	—	—	—	—
Baca	1	—	—	—	1	—	—	—	—	—	—
Kaffir	—	—	—	—	—	—	—	—	—	—	—
Hottentot ...	4	3	—	—	1	—	—	1	1	—	1
Griqua	1	—	—	—	1	—	—	—	—	—	—
Basutos—											
Basuto ...	11	1	1	—	3	—	1	—	—	2	2
M'Kalonga...	—	1	—	—	—	—	—	—	—	—	—
M'Naring ...	—	—	—	—	—	—	—	—	—	—	—
M'Gato ...	1	—	—	—	—	—	—	—	—	—	—
M'Nvali ...	—	—	—	—	—	—	—	—	—	—	—
Marolo ...	2	—	—	—	1	—	—	—	—	1	1
Bechuana ...	1	—	—	—	—	—	—	—	—	—	—
Baralong ...	1	1	—	—	1	—	—	—	—	—	—
Zulu...	30	1	1	3	22	1	1	4	—	—	5

TABLE XIII.—(*Continued*).

Tribe.	Ent-amœba coli.	Giardia intes-tinalis.	Chilo-mastix mesnili.	Tricho-monas intes-tinalis.	Spiro-chæta eury-gyrata.	Prowa-zekia cruzi.	Bodo stercor-alis.	Cerco-monas parva.	Isospora bigemina var. hominis.	"Free-living" Amœbæ.	Blasto-cystis hominis.
Swazi & Dwazi	3	1	1	—	3	—	—	—	—	—	—
Msutu	19	1	1	—	13	—	—	—	—	1	5
East Coast—											
Delagoa Bay	—	—	—	—	—	—	—	—	—	—	—
Tonga... ...	1	—	—	—	1	—	—	—	—	—	—
Shangaan ...	9	—	—	—	10	1	—	—	—	—	2
Portuguese East African	2	—	1	—	1	—	—	—	—	—	—
Moçambique	2	—	—	—	1	1	—	—	—	1	—
Blantyre ...	—	—	—	—	—	—	—	1	—	—	—
Nyambaan...	—	1	—	—	1	—	—	—	—	—	—
M'Chopi ...	1	—	—	—	1	—	—	—	—	—	—
Bantu (Uganda) ...	—	—	—	—	—	—	—	—	—	—	—
Nyasa	1	—	—	—	—	—	—	—	—	—	—
Somali	—	—	—	—	—	—	—	—	—	—	—
Indian	8	2	—	1	7	—	—	1	—	—	1
Chinese	—	—	—	—	1	—	—	—	—	—	—
TOTALS ...	129	15	6	7	93	4	2	10	1	8	24

XI.—DYSENTERIC STOOLS OF NATIVES.

The stools of 13 dysenteric Natives were examined, many of them daily, and in each case the infecting organism was *Entamœba histolytica*. Three cases were carriers, two were acute cases that rapidly proved fatal, the remainder being at first acute and then passing to the sub-acute form. Four Zulus, four Msutus, two Shangaans, one Somali, one Hottentot, and one Blantyre Native were infected.

These 13 cases were encountered during the ordinary course of the investigation. They may, perhaps, be regarded as affording some indication of the proportion of cases of amœbic dysentery which may occur ordinarily among the native population under relatively normal conditions.

XII.—GENERAL SURVEY OF THE HELMINTHES FOUND IN STOOLS OF NATIVES.

The most common helminthic infections detected in stools were those of the beef tape-worm, *Tænia saginata*, and the pork tape-worm, *Tænia solium*. Men having more access to meat than their fellows appeared to be more parasitised. Members of the tribes located from Cape Colony to the Transvaal formed the bulk of those whose stools were critically examined, the East Coast and Asiatic peoples being in the minority. However, when the results of infestation with helminthic parasites, as observed at post-mortem examinations, are considered, it seems correct to infer that the East Coast natives harbour more helminthic parasites than those of the Union of South Africa.

The number of times each Helminth was recorded is set forth in Table XIV. As no stool examined ever contained more than one kind of Helminth, the number of times each Helminth was recorded is the same as the number of individuals infected therewith, namely, 43, as will be seen from Table I, columns 4 and 5, or from Tables IV, VII, XI.

TABLE XIV.

Number of Times each Helminth was Recorded in Stools of Natives.

Tribe.	Tænia solium.	Tænia saginata.	Hymenolepis nana.	Schistosoma mansoni.	Oxyuris vermicularis.	Ascaris lumbricoides.
Cape	—	1	1	—	—	—
Pondo	—	1	—	—	—	—
Baca	—	1	—	—	—	—
Hottentot	—	1	—	—	—	—
Basuto...	2	1	—	—	—	—
M'Kalonga	—	1	—	—	—	—
Zulu	5	17	—	1	—	1
Msutu	—	—	—	—	—	1
Shangaan	2	1	—	—	1	—
Moçambique	1	—	—	—	—	—
Blantyre	—	—	—	1	—	—
Bantu	—	1	—	—	—	—
Indian	1	1	—	—	—	—
TOTALS	11	26	1	2	1	2

One kind of Helminth only was found in each Native stool (see p. 22).

Tape-worm infections, derived from improperly cooked beef and pork, form a large proportion of the total number of the Helminthes found in stools. Such occurrences direct attention not only to the infection among Natives, but to the necessity for proper disposal of human excrement on the one hand, and to systematic meat-inspection on the other.

XIII.—COMPARISON OF THE ENTOZOA FOUND IN NATIVE STOOLS WITH THOSE FOUND IN EUROPEAN STOOLS.

It was thought that it might be useful to compare the Protist and Helminth fauna of the stools of white men domiciled in South Africa with that found in Natives. For this purpose, a series of stools was examined from one of the European wards in the hospital, the same procedure being adopted as in the case of stools of Natives. The stools of men suffering from such maladies as dysentery and enteric fever are excluded from the results now tabulated, as the content of the intestine under such conditions is abnormal. Also, although a number of cases of amœbic dysentery among both white men and Natives was investigated, these are not included in the table, but are summarised in separate paragraphs. (See pp. 22, 27.)

The report on the first sixty stools of white men examined is now set forward on the same lines as for natives. The race given is in accordance with the statements of the men, the term " Colonial " being used for men born in South Africa, all of whom, it happened, were of mixed British and Dutch ancestry.

The distribution of the Entozoa detected in the stools of these various white men is set forth in Table XV, and further particulars regarding the occurrence of the organisms are summarised on the page following.

TABLE XV.

Distribution of Entozoa in the stools of various White Races examined.

Race.	Number examined.	Number infected.	Number of patients infected with Protista only.	Number of patients infected with Helminthes only.	Number of patients infected with both Protista and Helminthes.	Single protistan infections.	Double entozoal infections.	Multiple entozoal infections.
British	32	16	16	—	—	11	4	1
Colonial	8	5	5	—	—	4	1	—
Dutch	4	3	2	—	1	1	1	1
Russian Jews ...	14	7	7	—	—	4	3	—
German	1	1	1	—	—	1	—	—
Portuguese... ...	1	1	1	—	—	—	1	—
TOTALS	60	33	32	—	1	21	10	2

Infection with Entozoa thus occurred in 55 per cent. of the white men examined, as compared with 56·8 per cent. infection among the natives, of whom it must be remembered many more were examined. Helminthic infection was relatively rare—a result doubtless to be correlated with more attention to the cooking of food on the part of the white man.

Single infections with Protista occurred in twenty-one cases, each nationality, except the Portuguese, being represented.

Entamœba coli was present in the stools of eight British, three Colonial, one Dutch, and three Jews. *Spirochæta eurygyrata* occurred in three British, one Colonial, one Jew, and one German.

The ten double entozoal (Protist) infections were distributed among all the nationalities represented except the German.

Entamœba coli and *Spirochæta eurygyrata* co-existed in the stools of three British, one Dutch, three Jews, and one Portuguese. *Entamœba coli* occurred with *Blastocystis hominis* in one British, and *Spirochæta eurygyrata* together with "Free-living" Amœbæ was present in the fæces of a Colonial.

One multiple Protist infection only was found in a man of British race, the combination of parasites being *Entamœba coli*, *Trichomonas intestinalis* and *Spirochæta eurygyrata*.

The only mixed multiple infection, and also the only case in which helminthic parasites were observed, was in a man of Dutch descent, who harboured *Tænia saginata*, *Spirochæta eurygyrata*, and "Free-living" Amœbæ in his intestine, the last-named parasites being found in several successive stools, suggesting that they had become somewhat habituated to the intestinal environment.

The general occurrence of *Entamœba coli* and of *Spirochæta eurygyrata*, either separately or together, among men of various nationalities as well as among the different Natives of Africa and of India examined, shows the wide distribution of these organisms. While both parasites multiply more vigorously in an unhealthy intestine than in a healthy one, they appear to do relatively little or no gross harm to their hosts. These organisms, then, apparently have long been parasitic in man, and a mutual tolerance has become established between host and parasite, manifesting itself in non-pathogenicity on the part of the parasite.

Blastocystis hominis was found in the stools of one patient, and the said stool was markedly sour in smell and acid in reaction when this organism was detected. At first the Blastocystis was present in rather large numbers. As the acid reaction diminished, the Blastocystis also decreased, and when the reaction of the stools became markedly alkaline no Blastocystis could be found. In the cases of Blastocystis in Natives the parasite was usually most abundant in slightly acid stools, though also present in alkaline ones.

Dysenteric Stools of Europeans.

During the same period as the stools of men unaffected by intestinal complaints were examined, stools of ten dysenteric white men were examined. In each of these cases the excitant of the dysentery was *Entamœba histolytica*, and in most of the cases, the malady was of the acute type. The nationalities represented were three Colonials, two English, two Dutch, two Russian Jews, and one Greek. The parasites were usually observed in both the trophic and the encysted condition. It was relatively a heavy proportion of men infected, seeing that ten out of a total of seventy stools, all from one ward in the Johannesburg Hospital, contained *E. histolytica*. It is, however, probable that the percentage of infections is above the average, and that the number of sporadic cases among the total white persons under ordinary conditions is not so large. It may be mentioned that only one of the amœbic dysentery patients had been serving with the Forces, and his service had been in France for two years. He stated that, while in France, he had not suffered from any intestinal complaint, but had had several attacks of malaria and was sent back to South Africa after being gassed.

I may mention that, as noted on p. 19, I was also able to examine the stools of a non-hospital patient, a South African of Dutch descent, believed to be suffering from amœbic dysentery, but whose motions contained a pure infection of various stages of *Isospora bigemina* var. *hominis*. After the elimination of the parasite by appropriate treatment the man recovered.

SECTION B.—ENTOZOA FOUND AT POST-MORTEM EXAMINATIONS.

XIV.—DISTRIBUTION OF ENTOZOA IN NATIVES AS OBSERVED IN 104 POST-MORTEM EXAMINATIONS.

Many interesting observations on various Entozoa have accrued from the detailed examination of the alimentary canals of a total of 104 Natives who had died at the W.N.L.A. Hospital, Johannesburg, generally while awaiting repatriation. The intestine was examined minutely in each case, and scrapings of the mucosa made from the pylorus to the anus, thus ensuring that each region of the whole intestinal tract was subjected to microscopical examination. Fresh preparations were chiefly used, but stained ones were made when necessary, and material was also preserved for subsequent sectioning. Whenever pathological features were noted in connection with parasitic Protozoa, reference was made to medical pathologists at the Institute, and I wish to thank very heartily Drs. Watkins-Pitchford, J. G. Becker, and Lister, who came to my assistance, and from whose descriptions the pathological notes in this section are made.

It may be observed that rather different results were obtained as to entozoal infestation as observed post-mortem, from those derived from stool examinations, this being due in part to the different tribal distribution of the Natives examined *post mortem*. Of these latter, practically two-thirds were East Coast natives; the actual numbers were 69 out of 104 examined, the remaining 35 being Natives of other parts of South Africa. Fifty-four East Coast Natives out of the 69 were infected with Entozoa, and of the other South African Natives 25 were similarly parasitised. "Mine boys" live under different conditions from those prevailing among the Natives employed as labourers and house-boys. The condition of life when relatively segregated in large numbers in compounds may be conducive to the spread of Entozoa to some extent, but it seems more probable that the Natives are already infected when they reach the Rand than that they acquire many of the infections when resident there. The examination of the bodies of several "new recruits" for the mines has shown the presence of almost the same Entozoa as were present in men who had lived on various mines for some time. Age apparently had no significance in relation to parasitism, but, as the majority of the men were between the ages of 30 and 40, and very few were outside this range, it is hardly possible to give exact information.

Post-mortem examinations showed that practically 76 per cent. of the men were parasitised, the actual numbers being 79 out of 104. Infections with a single Protist, or with one kind of Helminth only, were comparatively few, as were double infections with Protista or Helminthes. On the other hand, the numbers of mixed double infections and of multiple infections, when as many as five or six separate genera of Entozoa were present in the same intestine, were greatly increased.

The tribal distribution of the deceased Natives in whom intestinal Entozoa were found *post mortem*, together with the general character of the infections and number of times of their occurrence, is set forth in Table XVI. It will be seen that there is a greater diversity in the types of infection than was present in the stool examinations.

Detailed analyses of the infections observed *post mortem* will be found in subsequent sections.

TABLE XVI.

Tribal Distribution of deceased Natives in whom Intestinal Entozoa were found Post mortem.

Tribe.	Number examined.	Number infected.	No. with Protista only.	No. with Helminthes only.	No. with both.	Single Protist.	Single Helminth.	Double Protist.	Double Helminth.	One Protist and one Helminth.	Multiple Entozoa.
M'Chopi... ...	24	20	3	4	13	1	2	1	1	5	10
Nyambaan ...	19	16	3	1	12	1	—	2	1	1	11
Shangaan ...	18	12	1	5	6	—	3	—	2	3	4
Blantyre... ...	2	2	1	—	1	—	—	1	—	1	—
Moçambique ...	3	2	1	—	1	—	—	1	—	—	1
Tonga	3	2	1	—	1	—	—	—	—	1	1
Bechuana ...	6	4	1	—	3	1	—	—	—	2	1
Msutu	7	5	1	—	4	1	—	—	—	1	3
Basuto	8	4	2	2	—	1	2	1	—	—	—
M'Xosa	5	4	1	—	3	1	—	—	—	1	2
Pondo	5	5	—	3	2	—	3	—	—	2	—
Baca	2	2	—	1	1	—	1	—	—	—	1
Fingo	1	0	—	—	—	—	—	—	—	—	—
Swazi	1	1	—	—	1	—	—	—	—	1	—
TOTALS ...	104	79	15	16	48	6	11	6	4	18	34

XV.—SINGLE PROTIST INFECTIONS OBSERVED *POST MORTEM*.

Infections with a single kind of Protist only were observed in six cases. Three genera—Entamœba, Giardia, and Spirochæta—were represented, and were distributed among six tribes as follows:—

TABLE XVII.

Parasite.	Tribe.	No.
Entamœba coli	M'Xosa	1
	Basuto	1
	Msutu	1
Giardia intestinalis	Nyambaan	1
	Bechuana	1
Spirochæta eurygyrata	M'Chopi	1

XVI.—SINGLE HELMINTHIC INFECTIONS OBSERVED *POST MORTEM*.

Infection with one species of worm only was observed in eleven cases, three genera of worms being represented. The distribution of the parasites is shown in the accompanying table.

TABLE XVIII.

Parasite.	Tribe.	No.
Ascaris lumbricoides	Basuto	2
	Shangaan	1
	M'Chopi	1
Tænia solium	Shangaan	2
Tænia saginata	Pondo	3
	Baca	1
Trichuris trichiura	M'Chopi	1

With the exception of Trichuris, the other parasites had been detected also in the investigations of stools. Although Trichuris was found in all at twenty-two post-mortem examinations (see pp. 30—38), yet one only of the infected men was other than an East Coast Native, the exception being a M'Xosa. This may explain the non-appearance of either Trichuris ova or worms at examinations of stools, as relatively few stools of East Coast Natives were examined.

XVII.—DOUBLE PROTIST INFECTIONS OBSERVED *POST MORTEM*.

Three groups of double Protist infections, distributed among five tribes, were observed in six cases, thus:—

TABLE XIX.

Double Protist Infections.	Tribe.	No.
Entamœba coli and *Giardia intestinalis*	Nyambaan	2
Entamœba coli and *Blastocystis hominis*	Blantyre	1
	Moçambique	1
Entamœba coli and *Spirochæta curygyrata*	Basuto	1
	M'Chopi	1

The presence of *Giardia intestinalis* was associated with an ulcerated condition of the intestine in each case, and in the inflamed areas it was the only parasite detected.

XVIII.—DOUBLE HELMINTHIC INFECTIONS OBSERVED *POST MORTEM*.

Four cases of infection with two kinds of worms were observed, the men concerned being free from Protista. The parasites present were as follows:—

TABLE XX.

Double Helminthic Infections.			Tribe.	No.
Tænia solium	and	*Trichuris trichiura*	M'Chopi	1
Tænia saginata	and	*Trichuris trichiura*	Shangaan	1
Ascaris lumbricoides	and	*Trichuris trichiura*	Nyambaan	1
Ancylostoma duodenale	and	*Trichuris trichiura*	Shangaan	1

The case of Ancylostomiasis showed emaciation and anæmia, and the intestine contained large numbers of the parasites. The certified cause of death was Pulmonary Tuberculosis and Silicosis.

XIX.—MIXED DOUBLE INFECTIONS OBSERVED *POST MORTEM*.

Eighteen cases of infection with one Protist and one Helminth were found, seven different associations of the parasites occurring, and ten tribes being represented, as shown in the following table:—

TABLE XXI.

Mixed Double Infections observed Post mortem.

	Combinations of Parasites.		Tribe.	No.
Cestoda and Protista	*Tænia solium*	and *Entamœba coli*	M'Chopi	2
	Tænia saginata	and *Entamœba coli*	Swazi	1
	Tænia saginata	and *Spirochæta eurygyrata*	Pondo	1
Nematoda and Protista	*Ascaris lumbricoides*	and *Entamœba coli*	Pondo	1
			Bechuana	1
			Msutu	1
			Shangaan	2
			Nyambaan	1
			Tonga	1
			M'Chopi	3
	Ascaris lumbricoides	and *Spirochæta eurygyrata*	Bechuana	1
	Trichuris trichiura	and *Entamœba coli*	Shangaan	1
			Blantyre	1
	Trichuris trichiura	and *Giardia intestinalis*	M'Xosa	1

The East Coast Natives, as judged by the distribution of these pure double and mixed double infections, seem very susceptible to worm infections. In some cases the number of worms observed was considerable, *Ascaris lumbricoides*, in particular, occurring in knots and bunches that appeared capable of causing considerable intestinal obstruction in some cases. In other cases as many as twenty heads of *Tænia saginata* have been recovered, both in the case of single, double, and multiple infections. *Trichuris trichiura* was usually found in the cæcum, and in a few cases these parasites (chiefly adults, but some ova) were found in numbers in the vermiform appendix.

XX.—MULTIPLE ENTOZOAL INFECTIONS OBSERVED *POST MORTEM*.

Multiple infections with Entozoa were relatively frequent in intestines of Natives examined *post mortem*. (See Table XVI, last column). The greater number were mixed Entozoa, only two cadavers containing a pure protozoal fauna of three genera, and one of four genera (see Table XXII), and one a multiple pure infection of three Helminths. In the remaining thirty cases, the parasites observed consisted of combinations of two Protists and one worm, one Protist with two worms, two Protists with two worms, three Protists with one worm, three Protists with two worms, four Protists with one worm, two Protists with three worms, and three Protists with three worms. (See pp. 35—38). More than six different genera of animal parasites living within the same intestine were not observed. Tables showing the combinations of parasites and the tribes, members of whom harboured the infections, are appended.

TABLE XXII.

Multiple Protist Infections observed Post mortem.

Combinations of Protista.		Tribe.	No.
Entamœba coli, Trichomonas intestinalis	and *Spirochæta curygyrata*	M'Chopi	1
Entamœba coli, Giardia intestinalis	and *Spirochæta curygyrata*	Shangaan	1
Entamœba coli, Giardia intestinalis, Trichomonas intestinalis	and *Spirochæta curygyrata*	M'Chopi	1

Multiple Helminthic Infections observed Post mortem.

One multiple helminthic infection only was observed. A M'Chopi was infected with *Ancylostoma duodenale*, *Ascaris lumbricoides* and *Trichuris trichiura*, the two former worms being numerous. The certified cause of death in this case was Pulmonary Tuberculosis.

Mixed Multiple Infections observed Post mortem.

Mixed multiple infections with Entozoa are perhaps best grouped according to the number of parasites present, three, four, five, and six different organisms thus being noted. The particulars are given in Tables XXIII—XXV, and on p. 38.

Multiple Infections of One Protist and Two Worms.

These infections were observed in nine cases, eight of whom had belonged to East Coast tribes. The frequency of worm infections in East Coast Natives is further emphasised by these results, which are listed in the following table.

TABLE XXIII.

Multiple Infections of One Protist and Two Worms.

Combinations of One Protist and Two Helminths.			Tribe.	No.
Entamœba coli, *Tænia saginata*	and	*Ascaris lumbricoides*	Nyambaan	1
			Shangaan	1
Entamœba coli, *Tænia saginata*	and	*Strongylus stercorea*	Nyambaan	1
Entamœba coli, *Schistosoma mansoni*	and	*Ascaris lumbricoides*	Nyambaan	1
Entamœba coli, *Ascaris lumbricoides*	and	*Trichuris trichiura*	M'Chopi	1
			Nyambaan	1
Giardia intestinalis, *Tænia saginata*	and	*Ascaris lumbricoides*	Nyambaan	1
Giardia intestinalis, *Tænia saginata*	and	*Trichuris trichiura*	Nyambaan	1
Spirochæta curygyrata, *Tænia solium*	and	*Ascaris lumbricoides*	M'Xosa	1

The infection with *Schistosoma mansoni* was very heavy, the whole of the large intestine being studded with small ulcers and bilharzial nodules, the latter containing both living and calcified ova. The infections with *Giardia intestinalis* showed ulcerative condition of the duodenum due to the flagellates, while the helminthic parasites were found in the ileum and cæcum of the cases respectively.

Multiple Infections of Two Protists and One Worm.

Multiple infections consisting of two Protists and one worm were observed in nine cases, these representing seven tribes. *Entamœba coli* was found in each of these cases, while in three cases parasitic Flagellata, representing three different genera, were present, the sites of intestinal infection showing inflammatory œdema. These mixed infections, with the tribes infested, are summarised in the appended table.

TABLE XXIV.

Multiple Infections of Two Protists and One Worm.

Combinations of Two Protists and one Helminth.		Tribe.	No.
Entamœba coli, *Spirochæta curygyrata*	and *Tænia solium*	M'Chopi	1
Entamœba coli, *Giardia intestinalis*	and *Trichuris trichiura*	Moçambique	1
Entamœba coli, *Trichomonas intestinalis*	and *Ascaris lumbricoides*	Baca	1
Entamœba coli, *Chilomastix mesnili*	and *Ascaris lumbricoides*	M'Chopi	1
Entamœba coli, *Spirochæta curygyrata*	and *Ascaris lumbricoides*	Bechuana	1
		Msutu	1
		Shangaan	1
		Nyambaan	1
Entamœba coli, *Spirochæta curygyrata*	and *Trichuris trichiura*	Shangaan	1

Multiple Infections with Two Protists and Two Helminths.

Multiple infections consisting of two genera of Protozoa and two Helminthes were obtained in four cases. *Entamœba coli*, *Ascaris lumbricoides*, and *Trichuris trichiura* were found in three of the cases, the second Protist differing in different cases, while *Ancylostoma duodenale* was associated with *Trichuris trichiura* in the remaining case. The observations are summarised in Table XXV.

TABLE XXV.

Multiple Infections of Two Protists and Two Helminths.

Combinations of Two Protists and Two Helminths.			Tribe.	No.
Entamœba coli, *Giardia intestinalis*, *Ascaris lumbricoides*	and	*Trichuris trichiura*	M'Chopi	1
Entamœba coli, *Trichomonas intestinalis*, *Ascaris lumbricoides*	and	*Trichuris trichiura*	Tonga	1
Entamœba coli, *Spirochæta eurygyrata*, *Ancylostoma duodenale*	and	*Trichuris trichiura*	M'Chopi	1
Entamœba coli, *Spirochæta eurygyrata*, *Ascaris lumbricoides*	and	*Trichuris trichiura*	M'Chopi	1

In connection with the case of Trichomonas infection, it is of interest to quote the pathologist's statement that "at the ileo-cæcal junction was a superficial ulcer, with edges slightly raised. The ulceration went through the mucous membrane down to the submucosa." In the ulcer and around it was thick greenish-grey mucus with tiny blood clots where Trichomonas was present. The certified direct cause of death in this case was Pulmonary Tuberculosis.

Multiple Infections with Three Protists and One Helminth.

Two multiple infections with three Protists and one Helminth were found *post mortem*; the Natives concerned belonged to the Transkei and the Transvaal. A M'Xosa harboured *Entamœba coli*, *Giardia intestinalis*, *Spirochæta eurygyrata*, and *Ascaris lumbricoides*, while *Entamœba coli*, *Spirochæta eurygyrata*, *Blastocystis hominis* and *Ascaris lumbricoides* were found in the intestine of a Msutu.

Multiple Infections with Five Entozoa.

Three forms of multiple infections comprising five genera of Entozoa were observed. Two genera of Protozoa were found in one case associated with three genera of Helminthes. Three Protists and two worms occurred in three cases, and four Protists and one worm were found in the intestine of one man. The tribal distribution and combinations of parasites were as follows:—

Two genera of Protozoa and three of Helminthes were present in one Nyambaan. The Protozoa were *Entamœba coli* and *Trichomonas intestinalis*, the Helminthes being *Schistosoma mansoni*, *Ascaris lumbricoides* and *Trichuris trichiura*.

Three different infections of three Protista and two Helminthes were found. One Nyambaan harboured *Entamœba coli*, *Trichomonas hominis*, *Spirochæta eurygyrata*, *Tænia saginata*, and *Ascaris lumbricoides*. Another Nyambaan was infected with *Entamœba coli*, *Spirochæta eurygyrata*, *Blastocystis hominis*, *Ascaris lumbricoides*, and *Trichuris trichiura*. The third case was a M'Chopi whose intestine was parasitised by *Entamœba coli*, *Trichomonas intestinalis*, *Spirochæta eurygyrata*, *Ascaris lumbricoides* and *Trichuris trichiura*.

One infection only of four Protista and one Helminth was detected, a Msutu being parasitised by *Entamœba coli*, *Trichomonas intestinalis*, *Spirochæta eurygyrata*, *Blastocystis hominis* and *Ascaris lumbricoides*.

The prevalence of helminthic infections among East Coast Natives is again noticeable.

Multiple Infection with Six Entozoa.

The presence of six Entozoa at the same time has only been observed once, when the intestine of a Nyambaan contained three Protists and three Helminths. The parasites were *Entamœba coli*, *Giardia intestinalis*, *Spirochæta eurygyrata*, *Tænia solium*, *Ascaris lumbricoides* and *Trichuris trichiura*.

At each point where Giardia were present other Protozoa were absent, as were also Helminthes, and these areas, in the words of one of the pathologists who kindly examined them for me, showed "inflammatory œdema."

XXI.—General Remarks Relative More Especially to the Presence of the Entozoa Detected *Post Mortem*.

In connection with the post-mortem examinations, about two-thirds of the Natives examined were East Coast boys, who form a large part (more than one-third) of the mine population of the Witwatersrand, and who seem recently to be more noticeable among the household servants than they were a relatively short time ago. Their cases, therefore, need more consideration than may appear at first sight, since it appears that there is an increase in the intimate relation of these Natives with South African households.

There is one obvious conclusion from the examination of the tables presented previously. The condition of parasitism is very prevalent among Natives. So prevalent is it, that a condition of mutual tolerance has often become established between the native host and the infecting organisms. The result of this tolerance is that, while the native host either may suffer little inconvenience personally or undergo intestinal disorders only at intervals, he becomes a potentially dangerous nuisance to non-immune hosts, and a disseminating centre for a number of gastro-intestinal troubles as a result of his promiscuous habits. An examination of the veldt in the vicinity of buildings in the course of erection will show only too readily the presence of human excrement. I collected a number of such samples for laboratory examinations and found chains of tapeworm proglottides, eggs of nematodes, cysts of various flagellates such as Giardia and Chilomastix, and on certain occasions—depending on the freshness of the stool—living Trichomonads, while I have also found cysts of *Entamœba histolytica* in such promiscuous evacuations. The excrement often swarmed with flies, and it is an established fact that certain worm eggs and numerous protozoal cysts can pass unchanged through the bodies of flies, whence they can easily reach food or water intended for human consumption. Samples obtained from shallow surface waters and spruits in certain localities have been found in the course of my examinations to be contaminated with cysts of flagellates, as well as with ova of Helminthes, and may thus serve as a source of infection both to man and to domestic animals. Also, in certain cases of infantile diarrhœa observed among Natives in the Johannesburg Hospital, I was able to obtain samples of the water used previously by the parents in preparing the food of the infants, and in several such samples there were flagellates (Trichomonas and Giardia) identical with those present in the motions of the children. Clean rats, whose food was mixed with this contaminated water, contracted flagellate diarrhœa and passed large numbers of the parasites in the fæces. Domestic vermin, such as rats and mice, and pests, such as cockroaches (see Sections X and XXIII, pp. 18 and 51) and flies, are additional agents or sources for the transference of parasitic diseases from man to man.

In this connection mention may be made of the use of night-soil for the purpose of soil fertilisation. Certain market gardeners, supplying their produce to the town, but living outside the Municipal boundary, use this material extensively, and not only is it emulsified and applied to the roots of plants, but a supply is sprinkled on the leaves of green vegetables such as lettuce. I have been able to recover cysts of parasitic flagellates (Giardia and Chilomastix) from lettuce leaves grown under the conditions just described. The cysts were mature and had not degenerated, and were fully capable of producing infection in the alimentary tract of man. As a matter of fact, in one case a persistent diarrhœa, due to *Giardia (Lamblia) intestinalis*, was traced to the consumption of such seemingly clean, but really contaminated, lettuce leaves. Great care, therefore, is necessary in the use of all salad food. The source of supply of such parasites is in the majority of cases to be found in the evacuations of Natives, and the number of infections found in stools and in the intestine itself examined *post mortem* shows, not only the frequency of their occurrence, but also their wide distribution among various tribes, the number of cases among the East Coast boys predominating.

The presence of numerous worms detected at post-mortem examinations also calls for attention. The presence of tapeworms and nematodes in human beings can be productive of bodily weakness, particularly among children. The commoner tapeworms, *Tænia saginata* from the ox and *Tænia solium* from the pig, seem very prevalent, and as many as twenty heads of *T. saginata* have been recovered from a single intestine. Such massive infection not only has its effects on the individual, but also shows the great possibilities of infection of cattle and antelopes and other game from pasture contaminated with the ova or segments of tapeworms derived from human sources. It also points to the necessity for *thorough* cooking of all animal food, and for the prevention of the access of animals used for food to garbage and excrement. The association of white children with native nurses who may be infected with Helminthes should also be strictly supervised and regulated.

XXII.—DIAGNOSTIC FEATURES OF THE ENTOZOA MENTIONED.

The diagnostic features of the various Entozoa mentioned in this paper are to be found in various standard works dealing with parasitology, such as "The Animal Parasites of Man," by Fantham, Stephens and Theobald, and "Manual of Tropical Medicine," by Castellani and Chalmers. As such books are not always available to South African practitioners and Medical Officers of Health, by request notes are appended here of the diagnostic features of the Entozoa more commonly encountered among the Natives of the Witwatersrand. The parasitic Protozoa will be first dealt with, and then the parasitic

Helminthes listed in the earlier part of this report. I wish to tender my hearty thanks to Professor H. B. Fantham for the use of his excellent illustrations of Protozoa, appearing on Plate I.

(I.) *Diagnostic Features of the Parasitic Protozoa mentioned in this paper.*

A.—*Parasitic Sarcodina.*

Entamœba histolytica and *Entamœba coli* are the organisms of this group that are represented.

Entamœba histolytica (Plate I, Figs. 1, 2), the causal agent of amœbic dysentery, is polymorphic. It occurs in freshly voided stools containing mucus, which stools are often blood-stained. Actively motile Entamœbæ move by the protrusion of processes (pseudopodia) of ectoplasm. The endoplasm is granular, and may contain red blood corpuscles, which are useful aids in the identification of *E. histolytica*. In acute cases, the motile feeding trophozoites are large, and the nucleus is often pale-staining in permanent preparations. A slightly smaller form of trophozoite, formerly termed *E. tetragena*, is found in subacute cases, and has a characteristic nucleus with a karyosome and centriole (Plate I, Fig. 1). The small precyst generation of trophozoites was formerly separately styled *E. minuta*. Multiplication is by binary fission, and occasionally by schizogony into four daughter forms.

Under favourable conditions encystment occurs; the round cysts (Fig. 2) produced measure about 10μ to 15μ in diameter, and have thin walls, though small strains with cysts varying from 7μ to 10μ in diameter are known. At first the cysts are uninucleate, but division into two, and finally four, occurs. In addition to the four nuclei, one or more dark staining masses, often termed "chromatoid" or "siderophile" bodies, are usually present in the cyst.

The transmission of *Entamœba histolytica* from man to man is usually brought about by the contamination of food or water with the infective cysts. The contamination may be direct, from excrement or sewage, or the cysts may be ingested by flies, passed unchanged through the bodies of the insects, and then be voided on fruit, milk, vegetable or other food. The "carrier" of amœbic dysentery, having become relatively immune for the time to the action of the parasite, is all the more dangerous to the community at large, and is also liable to severe recurrences of dysentery, should his general condition of vitality become lowered.

Entamœba coli (Plate I, Fig. 3), also parasitic in the human intestine, might be confused with *E. histolytica*. It is not pathogenic in its action, but multiplies more rapidly in a diseased than in a healthy intestine. The trophozoite is large, has a large round nucleus containing a fairly large karyosome, but no marked centriole. Its cysts, when mature, are double-walled, measure about 15μ to 20μ in diameter,

and occasionally a diameter of 30μ is attained. Chromatoid masses as a rule are absent from the cyst of *E. coli*, which contains eight nuclei when fully mature.

"*Free-living*" *Amœbæ*, such as *A. limax*, are occasionally found in human stools or in the human intestine. They are normally found in earth, or on vegetable matter, and are to be regarded, in the main, as temporary passengers in the human alimentary tract. The trophozoite stage is often large and vacuolated, while the nucleus has a large central karyosome. These amœbæ are sometimes placed in a separate genus, *Vahlkampfia*.

B.—*Parasitic Mastigophora*.

This large group includes the organisms, found in the intestine of man, which are responsible for flagellate diarrhœas, as well as others not yet definitely inculpated as possessing pathogenic properties.

Giardia (*Lamblia*) *intestinalis* (Plate I, Figs. 4, 5) has a definitely pathogenic action, as has been ascertained by animal experiments by Fantham and Porter (1916), as well as by numerous examinations of the human intestine *post mortem*. The flagellate form (Fig. 4) is an actively motile, pear-shaped, bilaterally symmetrical Protozoön, being leaf-like and pear-shaped when seen from above, and resembling a gondola when seen *en profile*. A large, concave sucking disc or cytostome is present on the under surface, and serves to attach the parasite to the intestinal epithelial cells, especially those of the duodenum. A central supporting rod or axostyle is present. Two large karyosomatic nuclei also are present, and two parabasal bodies are situated about the middle of the length of the axostyle. Four pairs of flagella occur, each flagellum having a small basal granule at its base. Two pairs of flagella are connected with the sucking disc, while the median ventral pair, which are the most active and prominent, originate near the axostyle below the cytostome, and the fourth pair form the terminal flagella. The organism is from 10μ to 21μ long, and from 5μ to 12μ broad. Multiplication by binary longitudinal and by multiple fission occurs in the flagellate stage. Resistant oval cysts, about 10μ to 15μ long, and 7μ to 9μ broad, ultimately containing four nuclei, the remains of the axostyle and the parabasal bodies, are produced (Fig. 5), and these constitute the infective stage, serving for the transmission of the parasite to new hosts.

Infection takes place by way of contaminated food and drink. Rats, mice, and cats can act as reservoirs of lambliasis, their excrement containing numerous cysts, while I have proved experimentally that the cysts can pass unharmed through the bodies of house-flies, blow-flies, and cockroaches, and are capable of re-infecting man.

Trichomonas hominis or *Trichomonas intestinalis* (Plate I, Fig. 6) is a pear-shaped organism with three free anterior flagella and an undulating membrane bordered by the lateral flagellum. An axostyle is present, and a large nucleus can be seen. The flagellate measures 10μ to 15μ by 5μ to 10μ. Rounded, contracted forms of the parasite

live a relatively long time in moist fæces. Food and water contaminated by infected human excrement, or by parasitised rats and mice, are the chief sources of infection.

Chilomastix mesnili (Plate I. Figs. 7, 8 and 9) is a pear-shaped organism allied to Trichomonas, but possessing a large cytostome about half the length of the body. Three anterior flagella are present, and a fourth, perhaps attached to a rudimentary undulating membrane, vibrates in the cytostome (Fig. 7). The flagellate measures about 14μ by 7μ. There is no axostyle. Encystment occurs, oval and lemon-shaped cysts (Figs. 8, 9) being produced. These are 7μ to 10μ long, and in addition to the nucleus the cytostome and vestiges of the flagella are found within the cyst. A vacuole containing a substance of the nature of glycogen may also be present. The cysts serve for transmission of the parasite by way of food and drink, and flies also act as transmitters.

Cercomonas parva (Plate I, Figs. 10, 11). A flagellate identical morphologically with *Cercomonas parva* was seen on several occasions. It agrees with the original description of *C. parva* given by Hartmann and Chagas (1910) from a case in South America, and with that of the same organism as observed by Fantham and Porter in cases from Gallipoli and Egypt. The presence of the organism in areas so diverse indicates it as a long-established parasite of man. The organism is pear-shaped, the body measuring 5μ to 20μ in length and 3μ to 5μ broad. A flagellum is present that traverses the length of the body and extends beyond it at both extremities. A single nucleus is present. Small, rounded cysts are produced, these being uninucleate (Fig. 11).

Prowazekia cruzi (Plate I, Figs. 12, 13) is a pear-shaped flagellate possessing two flagella, one anterior and one lateral trailing flagellum. The nucleus is large and round. A blepharoplast is present, and, near to it, the flagella originate from two contiguous basal granules, which often appear as one. The flagellate forms are polymorphic, a few being more or less sausage-shaped, others pear-shaped, and yet others rounded. The organisms possess a slight cytostome. The oval and pear-shaped forms measure 8μ to 12μ long, and they and the round forms are about 5μ broad. The few sausage-shaped forms seen by me measured about 12μ long and 5μ broad. Multiplication is by binary fission. Encystment occurs, the round or oval cysts measuring 5μ to 7μ in diameter (Fig. 13).

Bodo stercoralis, n. sp. (Plate I, Fig. 19). This organism is recorded from human fæces for the first time, so far as is known. The genus Bodo differs from Prowazekia in possessing no blepharoplast. *Bodo stercoralis* closely resembles *Prowazekia cruzi*, but has no blepharoplast, and the cytostome is shallow and inconspicuous. The body measures from 14μ to 19μ long and is from 6μ to 9μ broad. The nucleus is large with a distinct karyosome and well-marked nuclear membrane, on which six chromatoid granules are often deposited. Two small basal granules are present, from which the flagella originate.

The anterior flagellum is relatively stiff, the postero-lateral one is trailing and executes lashing movements. The postero-lateral flagellum is about as long again as the body. Oval cysts, about 6μ long by 5μ broad, each containing a nucleus of similar structure to that of the flagellate, are produced. This flagellate organism may possibly be connected with *Blastocystis hominis* (see below).

Spirochæta eurygyrata (Plate I, Fig. 14). This organism, as already noted by Fantham (1915, 1917), seems to be more numerous in a diseased than in a healthy intestine. The spirochæte has tapering ends, measures up to 15μ long, is about 0·25μ broad, and exhibits morphological variation. The number of its coils or waves varies with the rate of progression and the thickness of the organism. Resistant coccoid bodies are formed by *S. eurygyrata* and serve for the transmission of the parasite. The Spirochætes are not strictly Mastigophora, but form a group of their own, the Spirochætacea. They are considered here for convenience only.

Blastocystis hominis (Plate I, Figs. 15, 16), according to Alexeieff (1911), Brumpt (1912), and most workers, is of fungoid origin. The parasite appears as shining spheres, varying in diameter from 2μ to 15μ, which at first are uninucleate, with a film of cytoplasm surrounding a large vacuole. Binucleate stages are frequently seen (Fig. 15), as well as dividing forms (Fig. 16). At times, multinucleate forms are present, and still more rarely cysts containing numerous daughter spheres are found. Recently (1917) Chatton has stated that a Blastocystis which he has studied in geckos is a stage of a flagellate organism, having affinities with Bodo.

C.—*Parasitic Sporozoa.*

The chief Sporozoa parasitic in the human intestine are members of the Coccidiidea, namely, *Eimeria stiedæ* and *Isospora bigemina* var. *hominis*. Of these two organisms I have found Isospora both in stools and at post-mortem examinations. It is probable that *Eimeria stiedæ* will also be found parasitic in man in South Africa, as it is a fairly frequent parasite of the intestine and liver of rabbits here, among which it causes epizoötics.

Isospora bigemina var. *hominis* undergoes multiplication and sexual reproduction in the mucosa of the small intestine, the ileum often being most heavily infected. Sexual reproduction results in the formation of the infective oöcysts, which are voided with the fæces, and reach man by way of contaminated food or drink. These oöcysts (Plate I, Fig. 17) are somewhat lemon-shaped, are thin-walled, and are relatively frail in appearance. They measure 23μ to 33μ long, and are 11μ to 15μ broad. Each oöcyst, when mature, contains two oval sporocysts, 10μ to 13μ long by 8μ to 10μ broad. Four sporozoites differentiate within each sporocyst, and are grouped around a large residual mass. When such oöcysts are ingested by man, the sporozoites are liberated as small, vermicular bodies in the small intestine. They rapidly penetrate the epithelial cells in the jejunum and ileum, become round, and grow at the expense of the host cell, thus becoming

trophozoites. After a time asexual reproduction commences, 15 to 20 merozoites being produced from one schizont, and much epithelial destruction results. Schizogony is followed by gametogony, and after the union of the sexual elements, sporogony occurs. The infective oöcysts and sometimes epithelial cells containing trophozoites or schizonts are found in fæces and serve for diagnosis. I have proved experimentally that the oöcysts can pass unharmed through the alimentary canals of the house-fly, *Musca domestica*, and the green-bottles, *Lucilia caesar* and *L. serenissima*, which thus may act as carriers of Isospora.

Eimeria stiedæ (Plate I, Fig. 18), normally a parasite of hares and rabbits, is occasionally found in man. The oöcysts are oval, varying from 24μ to 49μ in length, and from 12μ to 28μ in breadth. Each oöcyst produces four sporocysts and each sporocyst contains two sporozoites. The life-history is similar to that of Isospora, and the infective oöcysts are diagnostic.

(II.) *Diagnostic Features of the Parasitic Helminthes mentioned in this paper.*

Since members of the Trematoda, Cestoda, and Nematoda have all been identified in the contents of the human intestine, they may be considered in the above zoological order.

A.—*Parasitic Trematoda.*

One genus of Trematoda has been detected in stools and in the human intestine, the parasite, *Schistosoma* (*Bilharzia*) *mansoni*, being first found in the form of the typical lateral-spined eggs or ova (Plate II, Fig. 8). At times portions of the adult worms were also found in fæces. The ova are oval, the lateral spine being situated nearer one pole of the body. The ova measure from 110μ to 165μ in length, and are from 60μ to 70μ broad. Mature living ova contain active miracidia, and when the ova are placed in warm water on a slide and examined under the microscope the miracidia show active vibrations of their cilia, and eventually force their way out of the eggs. A mature egg, as recovered from fæces, is shown on Plate II, Fig. 8. The adult worms are rarely found except at post-mortem examinations. Sometimes terminal spined eggs of *Schistosoma hæmatobium* (Plate II, Fig. 7) are found in fæces which have been contaminated with urine.

B.—*Parasitic Cestoda.*

Three forms of Cestoda are noted in this report, and they constitute some of the most widely distributed tapeworms. The pork tapeworm, *Tænia solium*, and the beef tapeworm, *Tænia saginata*, are probably better known than *Hymenolepis nana*, the cysticercus of which probably will be found in some insect such as a flea, while the adult tapeworm is very probably primarily a parasite of rats and mice.

Tænia solium and *Tænia saginata* may be detected as either scolices ("heads"), proglottides (segments), or as embryophores containing onchospheres (six-hooked embryos), incorrectly called eggs. The head in each worm has four cup-like suckers, and the segments measure from 9 to 35 mm. in length. The genital pores are lateral, and there is a median uterus with lateral branches.

Tænia solium (Plate II, Figs. 1, 4, 9).—The worm, when entire, measures usually 2 to 3 metres in length, but occasionally longer specimens occur.

The head or scolex is quadrangular, about one millimetre in diameter (Plate II, Fig. 1). It has a rostrum which bears two circlets of from 25 to 50 hooklets, one ring of which measures 160μ to 180μ, and the other 110μ to 140μ. The four suckers are rounded and conspicuous. The neck is fairly long and thin. The number of proglottides varies from 800 to 900.

The proglottides vary in size according to their position and age. Segments near the head are much broader than they are long, and about three feet from the scolex the length and breadth are equal, while after that they are longer than they are broad. The genital pores alternate fairly regularly. Sexually mature segments, after clearing, show the uterus with few, usually 7 to 10, lateral branches, these being often dendriform (Plate II, Fig. 4). Segments sufficiently mature for detachment are from 10 to 12 mm. long, and from 5 to 6 mm. broad. The mature segments often escape in chains, and do not exhibit active movements like those of *Tænia saginata*.

The "ova," really embryophores (Plate II, Fig. 9), are spherical, with a radially striated shell. Each measures 31μ to 36μ in diameter, and the onchosphere or hexacanth embryo, which is provided with six hooklets, is also spherical and is about 20μ in diameter.

Tænia saginata (Plate II, Figs. 2, 5, 10), the beef tapeworm, is larger than *T. solium*. It measures from 4 to 10 metres in length.

The head is pyriform to cubical in shape, and has neither rostrum nor hooks; it is 1 to 2 mm. in diameter. The suckers are hemispherical and are often pigmented. A small sucker-like organ replaces the rostellum (Plate II, Fig. 2).

The neck is long and about half the breadth of the head.

The proglottides number about 1,000, and are about 16 mm. to 20 mm. long by 4 mm. to 7 mm. broad when mature enough for detachment. They have been compared in appearance with melon and pumpkin seeds. The genital pores alternate fairly regularly. The uterine branches are numerous, 20 to 30 being present in a segment, and usually show dichotomy (Plate II, Fig. 5).

The embryophores are ovoid, 30μ to 40μ long by 20μ to 30μ broad (Plate II, Fig. 10). The shell is somewhat more transparent than that of *T. solium*, and the onchosphere has the three pairs of

hooks sometimes diverging somewhat more widely than those of *T. sclium*. However, in actual practice, it is not easy to distinguish between the embryophores of *T. solium* and *T. saginata*.

Hymenolepis nana (Plate II, Figs. 3, 6, 11) is a smaller tapeworm of man, being usually from 10 mm. to 40 mm. long and from 0·5 mm. to 0·9 mm. broad.

The head has a short, thick retractile rostrum, furnished with a single circle of 20 to 30 hooklets (Plate II, Fig. 3). The conspicuous suckers are about 0·08 mm. in diameter.

The proglottides are about 200 in number and are very narrow. They are from 0·4 mm. to 0·9 mm. in breadth and 0·14 mm. to 0·3 mm. in length. The genital pores are unilateral and marginal. The sexually mature segments contain three testes (a diagnostic feature), with a single vesicula seminalis, a branched ovary beneath which is a vitelline gland, and a large simple vagina (Plate II, Fig. 6).

The "eggs," or embryophores, are globular or oval with two distinct membranes, one of which is an uterine shell (Plate II, Fig. 11). The outer dimensions are 30μ to 60μ, the inner 16μ to 34μ. A mammillate projection may be present at each pole.

C.—*Parasitic Nematoda.*

The principal parasitic Nematoda observed in the stools or intestines of Natives are *Ancylostoma duodenale*, *Ascaris lumbricoides*, and *Trichuris trichiura*. I have also found *Oxyuris vermicularis* on one occasion.

Ancylostoma duodenale, or "hookworm," one of the chief causal agents of tropical anæmia, is a round worm with a cylindrical body tapering to the head. The anterior extremity is curved towards the dorsal surface of the body. The mouth is large and round, and two pairs of hook-like teeth are present on the ventral margin, and there is a small pair on the dorsal surface of the mouth. Two ventral chitinous plates are present in the floor of the mouth, and there is a prominent projection due to the opening of the dorsal head gland, this prominence being sometimes termed a tooth. Both male and female agree in the above particulars.

The male Ancylostoma is about 8 mm. to 11 mm. long by 0·4 mm. to 0·5 mm. broad. It has a bursa copulatrix at the posterior end, the bursa being umbrella-shaped and supported by chitinous rods. These rods or costæ are characteristic in their arrangement. In the median dorsal line is the dorsal costa, which bifurcates into two, each of which is trifid. Posterolaterally one root passes out, the single costa dorsalis externa. In front of the costa dorsalis externa is a broad root with three branches, these being termed the costa lateralis posterior, costa lateralis media, and costa lateralis externa respectively. The ventral portion of the bursa is supported by the bifid costa ventralis on each side. Two long copulatory spicules project through

the opening of the bursa. They may be as much as 2 mm. in length. The testis is tubular, the vesicula seminalis is oval, and a long cement gland is present.

The female Ancylostoma is about 12 mm. long, and the body ends posteriorly in a spike which may be broken off. The vagina opens about 1 mm. behind the middle of the length of the body. Two long tubular bodies uniting to form a single vagina constitute the female reproductive organs. The ovaries form oblique coils, and each is thickened at its distal end to form a uterus, the oviduct lying between the ovary and the uterus. The part of the uterus continuous with the oviduct acts as a seminal receptacle. The eggs or ova are oval, and thin shelled, the shell appearing to be single (Plate II, Fig. 12), though it is really double in contour. The eggs are about 60μ long by 40μ broad, and as found in freshly shed fæces show 2 to 4 blastomeres, as a rule. Larvæ are found only in eggs from old fæces, but at a temperature of from 25° C. to 30° C. an embryo is developed in about 48 hours.

For diagnostic purposes, slightly developed eggs are to be sought in fresh stools prior to treatment, eggs containing embryos are found in stools that have been kept, but the embryos are not so far developed as those of *Strongyloides stercoralis*, with which they might be confused. The Ancylostomes themselves are usually recovered from the fæces after treatment of the patient with thymol.

Infection occurs by way of contaminated soil. A rhabditiform larva issues from the egg, and after an ecdysis or moulting alters somewhat in structure and becomes the resistant, infective, filariform larva, which forms a new skin beneath the old one, but retains the old one as a loose covering. This filariform larva pierces the skin of man and ultimately finds its way to the alimentary tract, especially the jejunum, where development into the adult worm subsequently occurs.

Ascaris lumbricoides, or round worm of man. These worms are large. The males measure from 15 to 17 or even 25 cm. long, and the females may attain a length of 40 cm., though most are about 25 cm. long. The body varies in colour from greyish yellow to reddish yellow, has a striated cuticle, and is capable of considerable extension due to its elasticity. Two well-marked lateral lines are present. The head is small and has three oral papillæ or lips, which are finely denticulate. The dorsal papilla bears two sensory papillæ, and the other two papillæ each have one sensory papilla.

The male Ascaris has a curved posterior extremity provided with two marked spicules, each about 2 mm. long. The cloacal orifice is provided with 70 to 75 papillæ, seven pairs being post-anal.

The female has convoluted ovaries, and the genital aperture is in the anterior third of the body. The posterior end of the body is relatively straight and without spicules.

The eggs or ova (Plate II, Fig. 13) are elliptical, with a thick, transparent shell, which, in the case of the freshly laid egg, is invested with a gelatinous or albuminous coat forming a series of wave-like

protuberances, and may be bile-stained. The ova measure 50μ to 75μ long, and are from 40μ to 55μ broad. The ova when freshly deposited are unsegmented. The albuminous coat disappears on keeping the stool.

Trichuris trichiura, the "whip-worm," is a small worm having the anterior part of the body very long and thread-like, while the posterior part is much thicker, that of the male being spirally coiled. The anus is posterior.

The male measures from 35 mm. to 45 mm. long, and has a spicule about 2·5 mm. long, surrounded by a retractile sheath possessing numerous minute spicules.

The female measures 35 mm. to 50 mm. long, the anterior filiform portion of the body being about two-fifths of the total length.

The eggs or ova have a characteristic barrel-shaped or "tea-tray" contour, the thick shell being brownish in colour and perforated at the poles (Plate II, Fig. 14). The perforations are each closed by a light-coloured plug, and the egg covering then resembles a tea-tray in shape. The ova, as found in fresh fæces, have undifferentiated contents. The ova measure on the average 50μ long by 23μ broad.

Oxyuris vermicularis, or "seat-worm," is white in colour, having a striated cuticle which projects outwards from the anterior part of the body as flanges in the mid-dorsal and mid-ventral lines, lateral flanges also being produced. The mouth possesses three small retractile labial papillæ.

The male Oxyuris is small, measuring 2 mm. to 5 mm. long. The posterior end of the body is curved and shows six papillæ. A hook-like spicule 70μ long is present.

The female is larger, measuring up to 10 mm. in length. The vulva is situated in the anterior third of the body. The anus is about 2 mm. from the tip of the tail.

The eggs or ova are oval, asymmetric, with double contoured though rather thin walls (Plate II, Fig. 15). They measure 50μ to 55μ by 16μ to 25μ. When fresh, they contain clear, tadpole-like embryos, which become coiled and nematode-like later. The ova escape per anum, and are usually re-introduced into the host or conveyed to new hosts by the fingers, which become contaminated by scratching. Adult worms can also escape per anum, and are capable of re-infecting man.

XXIII.—ON THE EXISTENCE OF A "NORMAL" PROTISTAN FAUNA OF THE HUMAN INTESTINE.

The use of analogy is a dangerous procedure. Recently, possibly from motives of expediency, it has been alleged by Wenyon (1917) that a "normal protozoal fauna" of the human intestine exists because a bacterial flora has been recognised, which has been considered both non-pathogenic and universal. It is overlooked that the life processes of bacteria—many "bacteria" of a few years ago are now proved to

be fungoid in nature—are of a very different character from those of animals. The conditions under which many bacteria live are totally repellent to animal life, and the environment of the culture, in which many bacteria flourish exceedingly, often proves fatal to a Protozoön introduced therein.

Again, because flagellates have been found in the stools of patients not obviously suffering from intestinal affections, that is, in " carrier " cases, it is unwarranted to conclude that such flagellates are harmless. The effects of mass reactions always need consideration, and while the effect of a few organisms may be very slight, the consequences of their vigorous multiplication may be serious. It is unsound to ignore the presence of any unicellular organism that is dependent for its nourishment on the intestinal contents or the tissues of the body of man; more especially is this so in the cases of certain of them when their pathogenicity has been established, not from morphological considerations alone (as has apparently been inferred by Wenyon), but from a series of animal experiments, details of which were published in the *British Medical Journal* of July 29, 1916, which evidence, easily accessible in Egypt, has been ignored.

It is one thing to allege dogmatically that a natural intestinal fauna exists, but quite another thing either to prove such a hypothesis or to produce definite evidence in support of such a statement. From a consideration of the Protista found in many thousands of stools of Europeans examined in England, as well as those of the Natives detailed in this report, it seems to me that of all the Protist organisms found in the human intestine, two only can be regarded as of general occurrence, namely, *Entamœba coli and Spirochæta eurygyrata*. Even in these cases, in which a certain degree of mutual tolerance between host and parasite has been established, the presence of the organisms in large numbers serves as an indication of a more or less diseased condition of the alimentary tract. Such organisms as *Blastocystis hominis* have been regarded by some as so harmless that their presence can be totally disregarded. The evidence on which such an assumption rests is extremely scanty, and the frequent association of these organisms with stools of abnormal consistencies, odours, and reactions needs further investigation.

Other parasitic organisms such as *Giardia intestinalis*, *Trichomonas hominis*, and *Chilomastix mesnili* are definitely known to be pathogenic in their action. The work of Escomel (1913, 1917), Mello Leitao (1913), and others in South America, of Lynch (1915) in the United States, of Mathis (1914) and of Noc (1909) in Tonkin, of Chalmers and Pekkola (1918) in Khartoum, of Brumpt (1912) in France, and of Fantham and Porter (1916) in England, among others, has firmly established that these organisms can, and do, produce intestinal disorders that are sufficiently distressing and debilitating to warrant careful attention. The present work has shown that in no case were these flagellates detected in men whose motions were absolutely normal, and that in every case in which these parasites were discovered *post mortem*,

inflammatory œdema was manifested, and in some cases definite ulcers with overhanging margins had been produced. As a special memoir on flagellate diarrhœas is in course of preparation, the subject need not be pursued further here, but it is certain that to regard *Giardia*, *Trichomonas*, and *Chilomastix* as "normal" inhabitants of the human intestinal tract is both inaccurate and misleading. There is no doubt that the original source of these intestinal flagellates is to be found in parasites of domestic vermin, such as rats and mice, by whose infected excrement human food and drink are contaminated. Such an origin has been established by the work of many investigators in Italy, France, England, Tonkin, and many parts of South America. I can add to the list of hosts usually implicated in the transference of *Giardia intestinalis*, *Trichomonas intestinalis* and *Chilomastix mesnili* to human food those common pests of kitchens, the cockroaches, frequently found in bakehouses and similar establishments. By allowing these insects access to infected excrement and collecting and examining the fæcal masses voided by them, I have ascertained that they could ingest the cysts of these flagellate Protozoa, and that the latter passed unchanged through their bodies. The said cysts when fed to white rats with bread caused the rodents to contract a flagellate diarrhœa, and from the fæcal pellets of the rodents cysts again were recovered. Thus, cysts of Giardia, Trichomonas, and Chilomastix originally derived from man were transferred successively from man to the cockroach, thence to the rat, whence by way of contamination of cereal food by the fæces of the prowling rat, the parasite could again reach man.

The route of the flagellates associated with diarrhœa can also be a direct one, cysts from an infected person reaching another by way of sewage-contaminated water, or vegetable food similarly fouled. The effects of the parasites on the hosts are hardly consistent with normal conditions, nor can the parasites be considered normal occupants of the human intestine.

The widespread occurrence of Helminthes among Natives contributing to the Johannesburg population has been shown both by Dr. Turner's investigations (1910) and by my present results. It has been well established that such worms are harmful, and I do not think that anyone would venture to designate *Tænia solium*, *Ancylostoma duodenale* or *Schistosoma mansoni* as factors of a "normal" fauna of the human intestine. Since such fallacious statements are not applied to the Helminthes found in man, there is no reason why they should be applied to the Protista found in the body of man. Harmony between host and parasite may become established, but as soon as anything occurs to interfere in any degree with the balance thus established, the mutual tolerance breaks down, the true nature of the Protist asserts itself, the reaction on the part of the host is temporarily insufficient to re-establish the balance of power, and pathogenicity becomes apparent. The seemingly harmless "passenger" Protist under normal health conditions can become the virulent parasite under conditions somewhat removed from the normal.

Thus, it seems highly probable that a protozoal fauna of the human intestine, that is in any way comparable with the bacterial flora of that organ, does not exist except perhaps in the very limited form provided by *Entamœba coli* and *Spirochæta eurygyrata*; and, as before mentioned, large numbers of these organisms are an indication of intestinal disorder, and hence strictly cannot be regarded as normal.

XXIV.—GENERAL CONCLUSIONS.

The native population of the Johannesburg area is very heterogeneous in character, the chief contributing areas from which its fluctuating constituents are derived being the East Coast, Cape Province, the Transvaal, Basutoland and Natal. Among all these Natives endoparasitic infestation is common, both Protozoa and Helminthes being represented.

Infections with one, two or more Entozoa occur. Six kinds of parasitic organisms have been found simultaneously in a single intestine. Protista are common, but being microscopic are more easily overlooked than the larger, obvious helminthic parasites. Nevertheless, the mass action of minute Protozoa may be more serious in their effects on the host than the larger and obvious helminthic parasites.

The parasitic Protozoa of known pathogenic action that have been detected in the stools of Natives and of a few Europeans in Johannesburg include *Entamœba histolytica*, the excitant of amœbic dysentery; *Giardia intestinalis*, *Trichomonas intestinalis*, and *Chilomastix mesnili*, which are associated with various flagellate diarrhœas; and *Isospora bigemina* var. *hominis*, which can cause coccidial diarrhœa.

In a few cases, rarer parasites have been observed. These include species of Prowazekia, Bodo and Cercomonas, often considered not to be pathogenic, and "free-living" Amœbæ, which latter are transient occupants of the intestinal tract, their normal habitat being earth or vegetation.

Entamœba coli and *Spirochæta eurygyrata* are very widely distributed parasites, have long been habituated to life in the human intestine, and a state of mutual harmony more or less exists between them and their hosts. Nevertheless, it has been found that these parasites multiply more abundantly in an unhealthy intestine than they do in a normal one, and their presence in large numbers may serve as an indication of intestinal disorder.

The number of infections with *Entamœba histolytica* that have been observed can be considered as some index of the number of sporadic cases normally present among a population living under normal conditions, and indicate also the possibilities of amœbic dysentery occurring in epidemic form, should conditions of living lower the standard of health now prevailing. Under less favourable conditions of living, diseases now relatively quiescent and sporadic tend to become fulminating and epidemic. The main modes of transmission

of *E. histolytica* to man are by direct infection of food and water, and by indirect infection by way of flies. Carriers of cysts are serious sources of danger or potential danger to those persons with whom they associate.

The same remarks apply to the wide tribal distribution of such parasitic Flagellata as *Giardia intestinalis*, *Trichomonas intestinalis*, and *Chilomastix mesnili*. Each of these organisms is capable of producing flagellate diarrhœa, and also each is difficult of elimination from the alimentary tract when it has once become established there.

Distressing diarrhœa in adults, and some forms of "green" or "infantile" diarrhœa in children, in Johannesburg, have been traced to the presence of these parasites, and with their elimination under suitable treatment the diarrhœa has ceased. Post-mortem examinations of Natives and of subinoculated animals have established their pathogenic action, and it seems probable that many obscure intestinal troubles that are difficult of elimination may be due to these organisms. Natural reservoirs of these flagellates are found in such domestic vermin as rats, mice and cockroaches, whose infected excrement in cereal and farinaceous food is one source of infection, the cysts voided with human fæces which contaminate food being the other.

Sporozoa, such as *Isospora bigemina* var. *hominis*, have been proved to be pathogenic to laboratory animals such as cats and white rats, in which they may excite marked enteritis, sometimes with fatal results. In man, though they do not always produce acute symptoms, they have been found in conditions of severe diarrhœa, blood and mucus being present in the evacuations, as I have myself observed. The oöcysts pass unchanged through the alimentary canals of the house-fly, *Musca domestica*, and the green-bottles, *Lucilia cæsar* and *Lucilia serenissima*, which consequently may act as carriers of Isospora. Cats and rats may also act as reservoirs.

The danger of the promiscuous habits of some of the Natives, whose evacuations are so deposited as to contaminate water and foliage, as well as serving as an attraction to such transmitters or carriers as flies, is obvious. It seems very probable that infected Natives serve as reservoirs of disease. Preventive measures against soil pollution and fly breeding and fly activities need vigorous prosecution.

Helminthic infections are widely distributed among Natives. Eosinophilia and anæmia are common results of worm infestation, and, if only in a slight degree, lower the vitality of the infected person, whose resistance to other diseases is less marked in consequence. The elimination of worm infections needs attention, and it appears desirable that some attempt should be made to inculcate sanitary habits, both with respect to preparation of food and to disposal of sewage.

The numerous cases of infestation with *Tænia saginata* and *Tænia solium*, due to the use of imperfectly cooked beef or pork respectively, call for more attention to the proper cooking of meat and consequent destruction of the bladder-worm stages of these parasites. Further, the disposal of infected human excrement, in which tape-worm eggs

may be present, should be so arranged that there is no contamination of herbage on which pigs or cattle feed. By preventing the development of the eggs into bladder-worms in pigs or cattle, the development of tape-worm stages in man are rendered impossible, while the proper cooking of meat also aids in tape-worm eradication. The presence of Hymenolepis in man is the result of faulty protection of food from pollution by domestic pests, such as rats and mice, the fleas infesting these animals transmitting the bladder-worms to human food. The necessary preventive measures are obvious.

Nematode infections also are numerous. The anæmia due to *Ancylostoma duodenale* is too well known to need comment. As the infective filariform larvæ of Ancylostoma gain access from polluted soil to the skin of man, the prevention of soil pollution is essential. This, combined with energetic treatment of persons found to be infected, is necessary to prevent sporadic infections assuming an epizoötic form under favourable circumstances.

Ascaris lumbricoides and *Trichuris trichiura* are also common. A toxin has already been isolated from Ascaris, and the possible effect of masses of these worms in causing intestinal obstruction is obvious. The fact that they are common does not diminish the necessity for their elimination from the human body, and the said elimination is followed by improved health.

Infections with the Trematode, *Schistosoma mansoni*, were observed in a few cases, severe diarrhœic or dysenteric conditions being present in each case. The danger of pollution of water with infected fæces, and of thus infecting the water snails whence the Schistosome cercariæ can reach man, is obvious. Similar remarks apply to the pollution of water with urine containing the eggs of *Schistosoma hæmatobium*.

Thus, helminthic infections of man need elimination, and the old statement that no animal parasite is entirely harmless to man is as true to-day as it was many years ago, and neither Protozoön nor Helminth can be excepted.

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EXPLANATION OF PLATES.

All figures were outlined with an Abbé-Zeiss camera lucida, using 16 mm., 4 mm. or 2 mm. apochromatic or 1-12 inch achromatic objectives, with compensating oculars 8, 12 or 18 or Huyghenian ocular 2. The approximate magnification is given in each case.

Plate I.

Figs. 1, 2.—*Entamœba histolytica.*

Fig. 1.—*Entamœba histolytica.* Trophozoite showing nucleus with karyosome and centriole, and two ingested red blood corpuscles. x 1300.

Fig. 2.—*Entamœba histolytica.* Typical cyst with four nuclei and chromatoid body. x 2000.

Fig. 3.—*Entamœba coli.* Typical cyst with eight nuclei. x 2000.

Figs. 4, 5.—*Giardia (Lamblia) intestinalis.*

Fig. 4.—*Giardia intestinalis.* Flagellate form. x 2500.

Fig. 5.—*Giardia intestinalis.* Cyst. x 2500

Fig. 6.—*Trichomonas hominis* or *intestinalis.* Flagellate form. x 2000.

Figs. 7, 8, 9.—*Chilomastix (Tetramitus) mesnili.*

Fig. 7.—*Chilomastix mesnili.* Flagellate form. x 2500.

Figs. 8, 9.—*Chilomastix mesnili.* Cysts. Fig. 8, Oval cyst. Fig. 9, Lemon-shaped cyst. x 2500.

Figs. 10, 11.—*Cercomonas parva.*

Fig. 10.—*Cercomonas parva.* Flagellate form. x 2500.

Fig. 11.—*Cercomonas parva.* Cyst. x 2500.

Figs. 12, 13.—*Prowazekia cruzi.*

Fig. 12.—*Prowazekia cruzi.* Flagellate form. x 2400.

Fig. 13.—*Prowazekia cruzi.* Encysted form. x 2400.

Fig. 14.—*Spirochæta eurygyrata,* group showing morphological variation. x 1400.

Figs. 15, 16.—*Blastocystis hominis.*

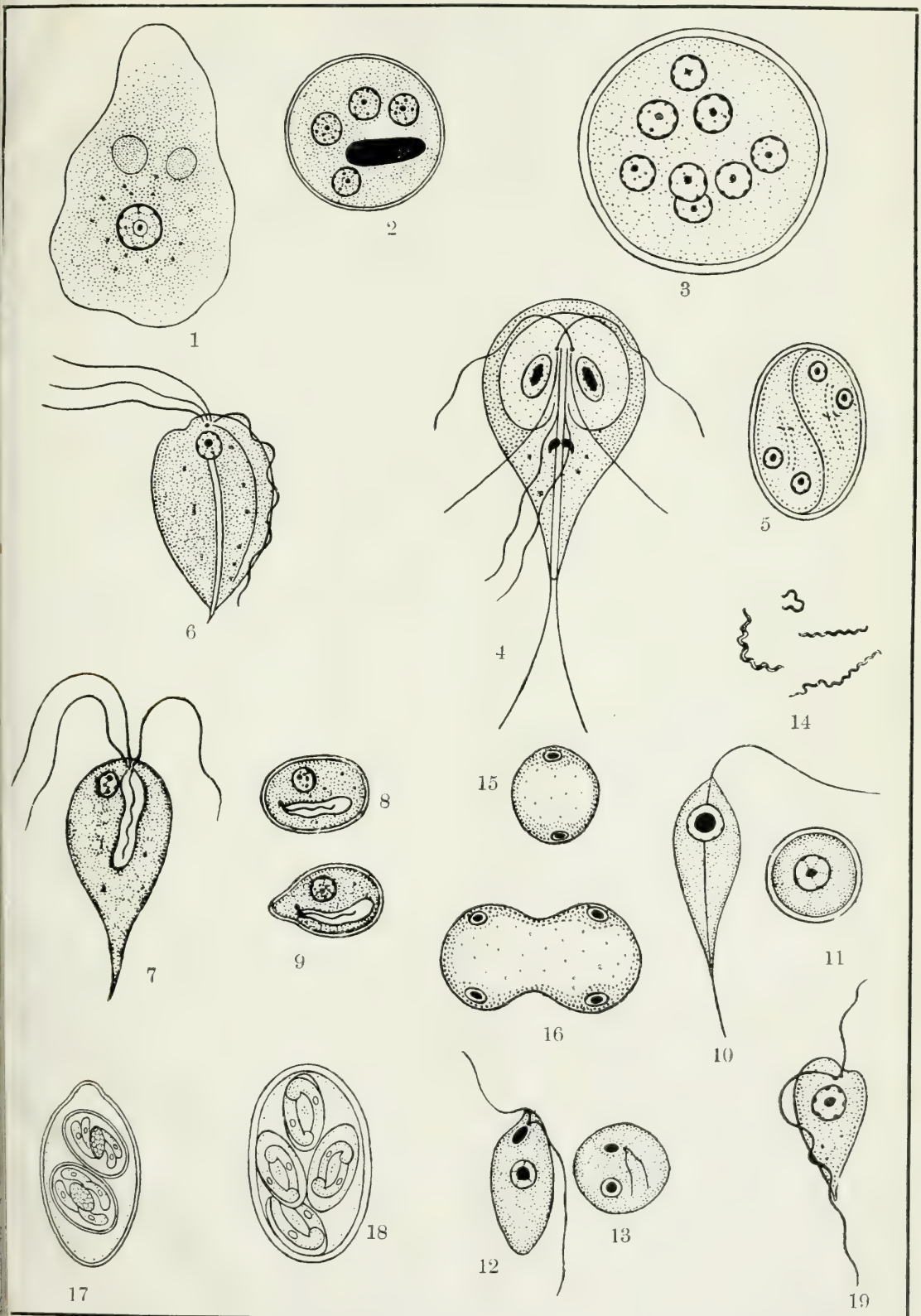
Fig. 15.—*Blastocystis hominis.* Typical binucleate form. x 1800.

Fig. 16.—*Blastocystis hominis.* Dividing form. x 1800.

Fig. 17.—*Isospora bigemina* var. *hominis.* Oöcyst containing two sporocysts each with four sporozoites. x 1000.

Fig. 18.—*Eimeria stiedæ.* Oöcyst containing four sporocysts each with two sporozoites. x 1000.

Fig. 19.—*Bodo stercoralis,* n. sp. Flagellate form. x 1500.



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EXPLANATION OF PLATE II

OVERLEAF.

PLATE II.

Fig. 1. —*Tænia solium.* Head showing suckers and hooks. x 45.

Fig. 2. —*Tænia saginata.* Head showing four suckers. x 25.

Fig. 3. —*Hymenolepis nana.* Head. x 75.

Fig. 4. —*Tænia solium.* Mature proglottis showing fully developed uterine branches. x 4.

Fig. 5. —*Tænia saginata.* Mature proglottis showing fully developed uterine branches. x 4.

Fig. 6.—*Hymenolepis nana.* Two proglottides showing three testes and one ovary per segment. x 80.

Fig. 7.—*Schistosoma hæmatobium.* From urine. Egg or ovum with terminal spine and miracidium inside. x 250.

Fig. 8.—*Schistosoma mansoni.* Egg or ovum with lateral spine. x 250.

Fig. 9.—*Tænia solium.* Embryophore. x 500.

Fig. 10.—*Tænia saginata.* Embryophore. x 500.

Fig. 11.—*Hymenolepis nana.* Globular embryophore with thick membrane and outer uterine shell. x 250.

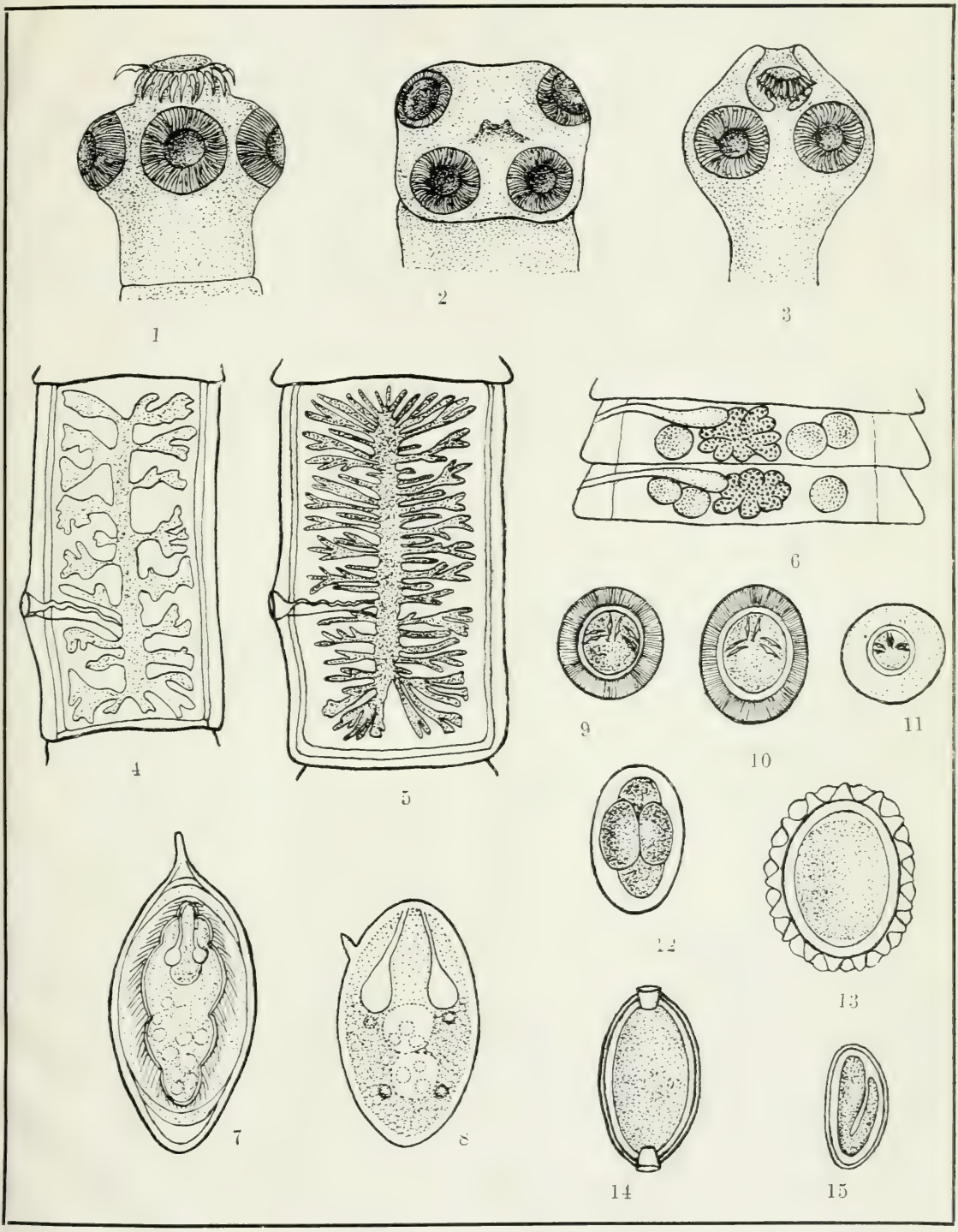
Fig. 12.—*Ancylostoma duodenale.* Egg, as found in fæces. x 350.

Fig. 13.—*Ascaris lumbricoides.* Egg, with gelatinous coat. x 450.

Fig. 14.—*Trichuris trichiura.* Tea-tray shaped egg. x 400.

Fig. 15.—*Oxyuris vermicularis.* Asymmetrical egg, with tadpole-like embryo. x 350.

OCTOBER 30TH, 1918.



A. Porter del.

OBSERVATIONS AND EXPERIMENTAL INVESTIGATIONS IN EPIDEMIC INFLUENZA.

BY

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I.

THE BACTERIOLOGY OF EPIDEMIC INFLUENZA ON THE WITWATERSRAND.

By F. S. Lister.

The pandemic of so-called "Spanish" Influenza first made its appearance on three of the gold mines of the Witwatersrand on September 22nd, 1918. Each of these mines is situated in the "Central Area," that is, in the town of Johannesburg itself. It is probable that isolated cases had passed unrecognized, amongst the community generally, earlier than this date.

The disease broke out on the above date with great suddenness, so that within twenty-four hours thousands of mine Native labourers and a number of Europeans were attacked on the three mines in question. It is not within my province to describe in detail the course which this epidemic subsequently pursued; it is sufficient to record here that it rapidly spread east and west of the central area until the whole of the mines were affected, and that the non-mining population of Johannesburg was not heavily involved until ten days or a fortnight later. Early in the epidemic the disease ran a mild course, but subsequently fatal complications became increasingly frequent and led to serious loss of life amongst both Europeans and Natives. The vast majority of deaths resulted from Broncho-pneumonia, due to infection by Pfeiffer's *Bacillus influenzæ*, together with other organisms.

The material investigated from a bacteriological point of view comprised: Eight swabs from the naso-pharynges of Natives attacked on September 22nd; nine samples of sputum from Natives attacked on the same date, but who did not show any particular signs or symptoms of serious lung involvement (at this period of the epidemic few cases of Broncho-pneumonia or Lobar Pneumonia occurred); six specimens of blood, cultured from four European women and two Natives respectively (all these six cases were well advanced in a serious form of the complicating Broncho-pneumonia; two of the Europeans and one Native eventually succumbed); one specimen of clear pericardial effusion from a Native who died; cultures from the pus of six cases of Empyema occurring during the epidemic at Kimberley and kindly sent to the Institute by Dr. Pearson, of that town; the lungs from 56 Natives who died on the gold mines; in addition to the above, eleven cultures of the Pneumococcus were handed to me by Mr. Joseph, one of the technical assistants at the Institute, six of these having been isolated from the lungs of patients who died and five from the sputum of cases suffering from "Spanish" Influenza with lung complications. Thus, in all, specimens from 97 cases were investigated. This material can be sub-divided into two classes—(*a*) swabs and sputum; (*b*) lungs, pericardial fluid, blood, and pus from empyemas. For the purposes of an investigation of the bacteriological aspect of the epidemic I regard the evidence obtained from the specimens enumerated under class (*b*) as far more valuable than that derived from class (*a*) specimens. My reason for this opinion is that the

organism responsible for this epidemic, and more particularly those organisms occurring in the fatal lung complications, are not uncommon inhabitants of the upper respiratory tract of man in ordinary times.

METHOD OF EXAMINATION OF THE VARIOUS SPECIMENS.

The material from swabs and sputum was smeared on slides in duplicate and each specimen was stained by Gram, and also by carbol-fuchsin (1-10); cultures from each specimen were obtained on human-blood-agar, and the resultant growths of micro-organisms examined as to the appearance of the colonies and microscopically by means of stained preparations.

The lungs and the pericardial fluid were subjected to direct microscopical examination by the method described above, and cultures, obtained upon human-blood-agar, were investigated. In a number of instances guinea-pigs and mice were inoculated with material from the lungs. Cultures of the Pneumococcus were tested with ten specific antisera for group identification; not all cultures were so tested, however, but no selection was made.

The blood cultures were made by withdrawing 5 c.c. of blood from the median basilic vein, 2.5 c.c. being transferred to each of two 50 c.c. bottles of trypsinated bullock's-heart-broth.

The pus from the six cases of Empyema was planted out at Kimberley by Dr. Pearson, on blood-agar, the culture tubes forwarded to the S.A. Institute for Medical Research, and the organisms present examined. The eleven cultures of the Pneumococcus handed to me by Mr. Joseph were all tested for group identification against the specific antisera.

RESULTS OF EXAMINATION OF CLASS (A) MATERIAL (SWABS AND SPUTUM).

As was to be expected, both direct microscopical and cultural examination showed the presence of a variety of micro-organisms. The Pneumococcus, *Micrococcus catarrhalis*, *Staphylococcus* (both *albus* and *aureus*) and diphtheroids being the most prevalent. A Gram-negative organism growing in fine colonies and corresponding morphologically with Pfeiffer's *Bacillus influenzæ* was observed in the cultures from three swabs and three sputa. However, nothing really consistent was forthcoming, and as deaths from Broncho-pneumonia now became very frequent, I turned my attention to the bacteriological examination of infected lungs obtained *post mortem*. The lungs were in every instance from Native mine labourers who, with few exceptions, worked on the Crown Mines (gold).

RESULTS OF EXAMINATION OF CLASS (B) MATERIAL (LUNGS, PERICARDIAL EFFUSION, BLOOD, AND PUS FROM EMPYEMA).

The following table shows the results of bacteriological examination of the lungs from fifty-six fatal cases:—

TABLE I.

THE BACTERIAL CONTENT OF THE LUNGS FROM FIFTY-SIX FATAL CASES.

No. of Cases.	Bacillus influenzæ present in	Pneumococcus present in	Micrococcus catarrhalis present in	Staphylococcus aureus or albus present in	Bacillus of Friedlander present in	Tubercle Bacillus present in	Streptococcus present in	Unidentified organisms present in
56	53	42	9	9 7 aureus 2 albus	2	2	2	3

It will be seen from the above table that the *Bacillus influenzæ* was present in 53 of the 56 cases examined, and that the Pneumococcus was by far the next most common organism. The remaining organisms were frequently present in quite small numbers, only a few colonies (in one instance only two) appearing on blood-agar. The two cases presenting the tubercle bacillus were both complicated by *Bacillus influenzæ*, and, in addition, with *Staphylococcus aureus* in one instance, and with Pneumococcus in the other. In four cases the *Bacillus influenzæ* was recovered in pure culture. It was very noticeable that the *Bacillus influenzæ* was frequently stained in a bipolar manner by dilute carbol-fuchsin, and also that this organism was more easily identified in lung tissue by this stain alone than by the same stain when briefly applied as the counter-stain in Gram's method.

PERICARDIAL EFFUSION.

This fluid proved to be sterile in the single specimen examined.

BLOOD CULTURES.

Blood was obtained from four European women and two Natives in the acute stage of their illness and was transferred to trypsinated broth—5 c.c. of blood to 100 c.c. of broth. In only one instance was any growth obtained; this was in the case of a European woman who ultimately recovered after a desperate illness. This organism proved to be Pneumococcus of group "B" (Type II. Rockefeller). Of the six cases referred to, two Europeans and one Native subsequently died.

PUS FROM SIX CASES OF EMPYEMA OCCURRING AT KIMBERLEY.

The six cultures derived from the pleural pus of Europeans are of considerable interest, in that three of them proved to be almost pure cultures of *Streptococcus hæmolyticus*; of the remainder, one was a pure culture of *Staphylococcus albus*, one a mixture of *Bacillus influenzæ* and Pneumococcus, whilst one was unsuitable for investigation owing to contamination.

The group of hæmolytic streptococci has come into great prominence within the last few years, especially in America, where epidemics of Broncho-pneumonia associated with these microbes have occurred on a large scale in many of the Army camps, and have caused a far higher case-mortality than is usually associated with pneumococcal Lobar Pneumonia. These infections, moreover, gave rise to a very high percentage of empyemas. Hæmolytic streptococcal empyemas ended fatally in numbers altogether out of proportion to the fatality of the same condition when due to the Pneumococcus. The literature on the subject is already extensive, and the *Journal of the*

TABLE II.

Group-classification of Pneumococci isolated.

Origin of Cultures.	No. examined.	Groups identified.										
		A.	B.	B Sub-groups.	C.	D.	E.	F.	G.	H.	J.	K.	Unclassifiable.
Lungs, European ...	2	—	—	1	—	—	—	1	—	—	—	—	—
Lungs, Native ...	28	—	—	5	—	1	8	1	2	1	1	2	7*
Sputum, European ...	5	—	—	2	—	—	2	1	—	—	—	—	—
Sputum, Native ...	1	—	—	1	—	—	—	—	—	—	—	—	—
Blood Culture, European	1	—	1	—	—	—	—	—	—	—	—	—	—
Blood Culture, Native	0	—	—	—	—	—	—	—	—	—	—	—	—

* Three of these cultures were not tested for sub-group B.

American Medical Association for the last two years will well repay perusal of the many articles dealing with this infection which have appeared during that period. In all the specimens examined from cases in Johannesburg I did not once observe hæmolytic streptococci, although the possibility of their occurrence was always before me, not only in view of the American experience, but also on account of an early report from the Capetown bacteriologists to the effect that a streptococcus (whether hæmolytic or not was not stated) was being very frequently found there in connection with the epidemic. It would be of the greatest epidemiological interest to know whether hæmolytic streptococcal infections were prevalent at Kimberley and Capetown during the recent epidemic. The unduly high death-rate at these two centres would, in such circumstances, be to some extent explained. It would also be of interest to know whether Measles was unduly prevalent, either shortly before, or during the epidemic, because hæmolytic streptococci are at times associated with Measles. The following table (II.) records the groups of the Pneumococcus identified by agglutination methods applied to 37 cultures of this organism derived from influenzal subjects.

The results of the tests shown in Table II. reveal two noteworthy peculiarities: 1. The absence, with one exception, of the common Lobar Pneumonia epidemic strains of the Pneumococcus (which do not normally exist in the upper respiratory tract) viz.: "A," "B" and "C"; and 2. The large proportion of the cases which were infected with group "E" (*Pneumococcus mucosus*) and sub-groups of "B," both of which varieties are quite frequently present in the upper respiratory tract of normal individuals.

Leaving out of consideration the six cultures from sputum there were 30 cultures derived from infected lungs and one from the blood. Twenty-four of these belonged to one or other of the classifiable groups.

ANIMAL INOCULATIONS.

Ten guinea-pigs were inoculated intraperitoneally with material from the lungs of ten cases. Only three of these animals died, and these were young animals, not quite half-grown; in the peritoneal fluid of two of them the *Bacillus influenzæ* was present, together with *Staphylococcus aureus* and Pneumococcus; the only organism recovered from the blood was the Pneumococcus. The peritoneal exudation of the third guinea-pig showed a Gram-positive cocco-bacillus which was not identified, whilst cultures from the heart blood showed *Bacillus influenzæ* and the unidentified cocco-bacillus which was present in the peritoneal exudate.

A number of mice were inoculated intraperitoneally with material from lungs but with such varied results as to be of little value for investigation. A number did not die, whilst others exhibited several

varieties of organisms in the blood and peritoneal exudate, and thus failed to serve, in this instance, as a differential *in vivo* culture medium.

Five rabbits were inoculated intravenously, each with a whole blood-agar culture of *Bacillus influenzæ* emulsified in 1 c.c. of ·85 per cent. saline. One of these cultures was obtained from the cerebro-spinal fluid of a case of Influenzal Meningitis, but this, as also the others, proved nonlethal. The four other cultures had been isolated from the lungs of cases which had succumbed to Broncho-pneumonia.

DISCUSSION.

The consistent presence of *Bacillus influenzæ* (Pfeiffer) in the lungs of patients who succumbed is strong evidence that this epidemic was due to this organism. Nevertheless, ever since Pfeiffer declared *Bacillus influenzæ* to be the cause of these epidemics, it has been customary, in many quarters, to make the reservation that some undiscovered, ultramicroscopic or filterable, virus may yet be found responsible, and that *Bacillus influenzæ* may only play the role of a secondary invader. Whilst this attitude may be a reasonable concession to prudence, the question at issue is, *at the present time*, of no great practical importance as regards the devising of means for combating the existing pandemic.

The bacteriological findings here recorded point to the disease being due to a primary infection with *Bacillus influenzæ*, which lowers the resistance of the individual, in many cases to such a degree as to leave him a prey to the attacks of several varieties of micro-organisms which normally exist harmlessly in his upper respiratory tract, in this instance, usually the Pneumococcus. Other organisms which I have referred to as being present in the lungs were frequently present in but small numbers. Very little is known as to the stage of the disease at which these secondary infections occur, and the question of agonal infection cannot be altogether dismissed with regard to some of the organisms concerned.

That Broncho-pneumonia can be caused by a pure infection with *Bacillus influenzæ*, and that it can terminate fatally, is suggested by the four cases referred to previously.

With regard to vaccine prophylaxis or therapy—so little is known that the question is hardly worth while discussing. Many fundamental facts in connection with the immunological possibilities of *Bacillus influenzæ* should be carefully worked out before anything can be expected in the way of prevention or treatment of this disease by such methods. The endeavour to prevent by means of vaccines the complications due to secondary infection is tantamount to putting the cart before the horse, and invites disappointment. Wholesale inoculations, with a view to preventing or treating this disease, *in the midst of an epidemic*, and in face of our lack of knowledge of the subject, appears to me to be quite unjustifiable.

CONCLUSIONS.

The epidemic disease prevailing in Johannesburg from September 22nd, 1918, and onwards, was apparently due to the *Bacillus influenzæ* (Pfeiffer).

The most frequent secondary invader was the Pneumococcus belonging to those groups which are existent in the upper respiratory tract in normal individuals.

Streptococcus hæmolyticus was recovered from three out of six specimens of pus from cases of post-influenzal empyema at Kimberley.

December 4th, 1918.

II.

EXPERIMENTAL INVESTIGATION OF EPIDEMIC INFLUENZA AT DURBAN.

By F. S. Lister and E. Taylor.

This work was carried out after the epidemic had swept through the country and only small secondary outbreaks of the disease were occurring in widely separated districts.

A recrudescence of Epidemic Influenza amongst the European population of Durban afforded the opportunity for conducting these experiments. The period of actual investigation extended from February 27 to March 27, 1919, inclusive.

The experiments to be described were performed upon eleven human subjects (Europeans) and four Cape Vervet monkeys (*Cercopithecus lalandi*).

The eleven men volunteered, in answer to advertisements published in the Johannesburg newspapers. Certain pecuniary terms were offered, including a free life-insurance available until one month after the termination of the experiment. Throughout this report the men will be designated by the numbers 1 to 11, and the monkeys by the letters A, B, C, and D.

None of the volunteers admitted having previously had an attack of Influenza. With the exception of Nos. 6 and 9, it is almost certain that all had been exposed to infection; in the case of No. 4, eight people living in the same house contracted the disease. No. 5 was closely associated by attendance on Influenza patients during the epidemic of October and November, 1918.

The men gave their ages as follows:—No. 1, 32 years; No. 2, 27 years; No. 3, 25 years; No. 4, 27 years; No. 5, 26 years; No. 6, 35 years; No. 7, 35 years; No. 8, 25 years; No. 9, 29 years; No. 10, 25 years; No. 11, 34 years.

Ten of these men (one volunteer was a resident of Durban) were taken from Johannesburg to Durban by rail, a distance of 519 miles, and were isolated on Salisbury Island. This island is situated in Durban Bay. Only a few families reside upon the island, but there is free access to and from the mainland (about a mile distant) by means of motor- and sailing-boats. There were no cases of influenza amongst the residents on the island. The men were housed in the Quarantine Hospital, which was empty at the time of their arrival; apart from the Superintendent in Charge and the Native servants they were the only occupants during the whole of the experimental period.

The monkeys were kept on the mainland at No. 3 General Hospital (Military).

FILTRATION EXPERIMENTS.

These experiments were designed to seek for a filter-passing organism as the cause of Epidemic Influenza. The procedure adopted was as follows:—typical cases of Influenza were selected from a number under treatment at the Municipal Beach Hospital. This Hospital admitted Influenza cases only. A nasal washing of approximately 70 c.c. of distilled water, together with some sputum, was taken from each case; the sputum was expectorated immediately after the performance of the nasal washing. The specimens of nasal washings and sputum from the three cases selected were placed in separate sterile bottles with sterile glass beads and thoroughly shaken for about five minutes. A portion of the fluid was then poured into tubes and submitted to centrifugalization by means of a hand-power machine for about three minutes, and the supernatant fluid was at once passed through a Berkefeld filter candle "N" under a positive pressure of 50 lbs. One c.c. of the filtrate from each of the three specimens was planted into broth and also on to blood-agar. The results were sterile in each case, but ærobic cultivation only was undertaken. These manipulations were executed at the Beach Hospital, on the mainland. The three filtrates and the remainder of the three unfiltered specimens were at once transported to Salisbury Island. The various specimens were here either instilled or sprayed into the noses and throats of the volunteers, and an hour later the four monkeys were treated in a similar manner. The material used for both the men and the monkeys was taken from the same bottle. The first experiment of this type dealt with the specimens from two cases of influenza, and the second, on a subsequent date, with material from a third case. A more detailed description of these two experiments may now be given.

Experiment I.

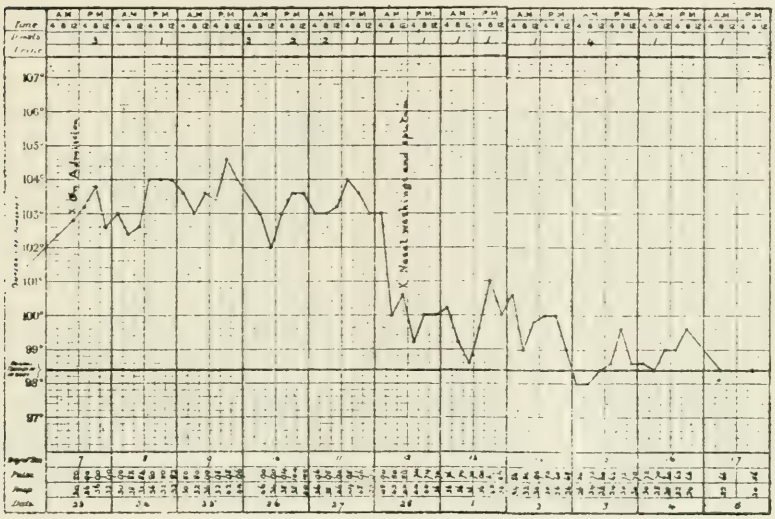
The first case of Influenza selected by us from which to obtain material for transference to human volunteers was that of Mr. "X", aged 20 years, a clerk; he eventually recovered.

A number of people living in the same house were attacked with the disease; first, three children; next, the mother and this patient; and the following day, two sisters, one of whom died in the Beach Hospital on the morning that nasal washings were secured from Mr. "X". These facts, taken in conjunction with the clinical findings, left little doubt as to the correctness of the diagnosis in this case.

This patient had a very severe attack with pulmonary complications; at one time it was thought that his recovery was unlikely, but at the time of the nasal washing his general condition had greatly improved. We realized that the disease in this instance was in a somewhat late stage, viz., *the twelfth day*; the case was, however,

specially suitable in other respects, and as nothing is certainly known about the period of infectiousness we decided to transfer material from this patient to four of our volunteers and to two monkeys. The temperature chart of this patient is exhibited below.

Chart of Mr. "X."



On February 28 nasal washings and sputum were obtained and treated as already described, the result being that we had two bottles one containing the diluted unfiltered secretions from this patient, and another the filtrate of the same material through a Berkefeld "N" Candle. Having inoculated blood-agar slopes, and broth, with material from each of these bottles, we at once proceeded to Salisbury Island, and within 1½ hours of the nasal washing we had transferred material to four of the volunteers in the following manner:—

No. 5 and No. 6.—In the case of each of these men 1 c.c. of the unfiltered mixture of nasal washings and sputum was instilled with a pipette into the nasal cavities and mouth, with the chin elevated.

No. 7 and No. 8.—In the case of each of these men 5 c.c. of the filtrate was instilled with a pipette into the nasal cavities, and the mouth.

None of these four men developed the disease; they were kept under observation for fourteen days, and were then submitted to another experiment to be described later.

The blood-agar and the broth inoculated with the filtrate remained sterile, and *Bacillus influenzæ* was not observed in the cultures derived from the unfiltered material.

Within an hour of the completion of the instillations in the men, two monkeys (*Cercopithecus lalandi*) were similarly treated, as follows:—

Monkey A, with 1 c.c. of the unfiltered material.

Monkey B, with 5 c.c. of the filtrate.

Neither of these monkeys exhibited any indication of infection; their temperatures were recorded daily for eight days and on no occasion showed any variation beyond apparently normal limits for these animals.

CHART A (MONKEY).

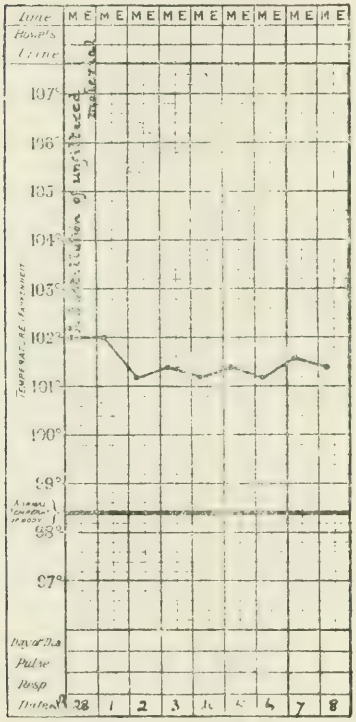
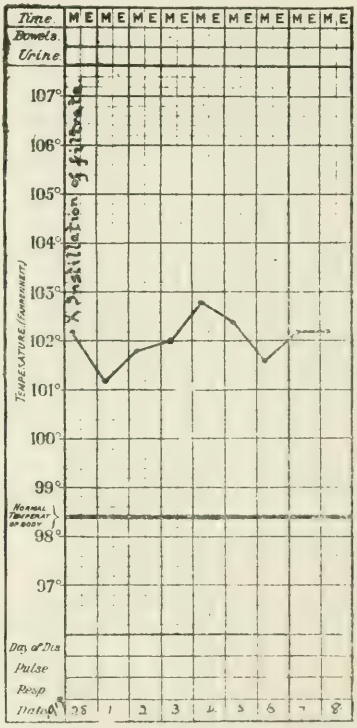
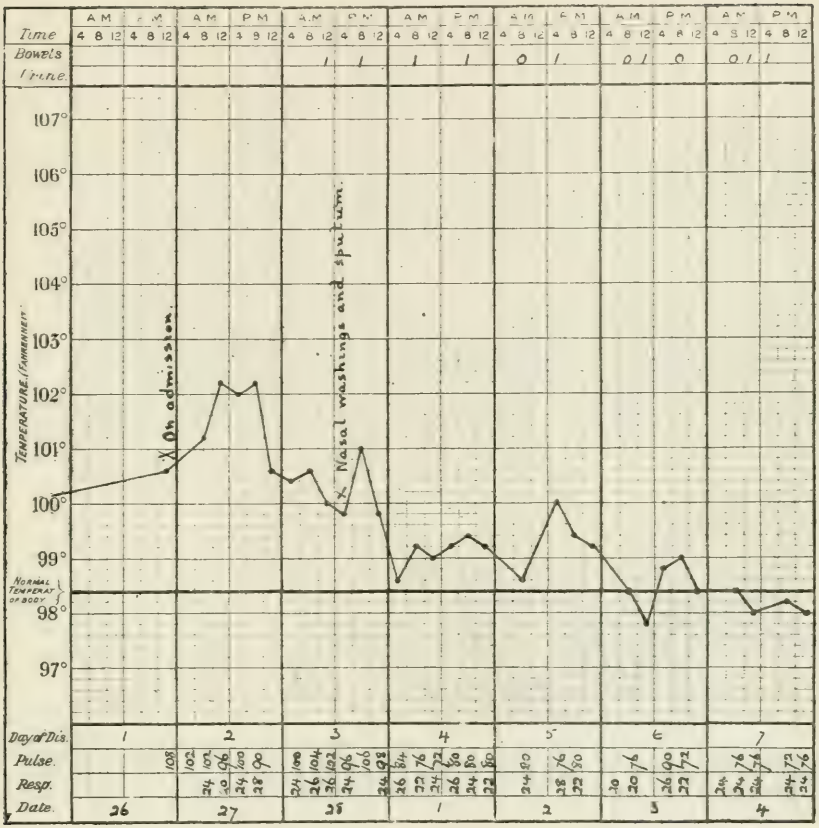


CHART B (MONKEY).



On February 28 a second case of influenza was also utilized in a manner identical in every respect with that detailed above, save that the material was derived from a patient at an earlier stage of the disease, viz., *the third day*. Sister "Y", who commenced nursing at the Beach Hospital on February 23, had developed an attack of Influenza forty-eight hours after spending a considerable time in controlling the previous patient, Mr. "X", who was at that time delirious. She suffered from a clinically typical but mild attack. Her temperature chart is exhibited below.

Chart of Sister "Y."



Very little sputum was obtainable, and it is questionable whether the expectoration was derived from the lungs; the patient, however, had experienced slight pain in the chest beneath the sternum.

The filtrate and the unfiltered material were cultured as in the previous case, the former remaining sterile, and the latter failing to show *Bacillus influenzæ*.

Within 1½ hours of the nasal washing, the filtrate and the unfiltered material was transferred to four volunteers as follows:—

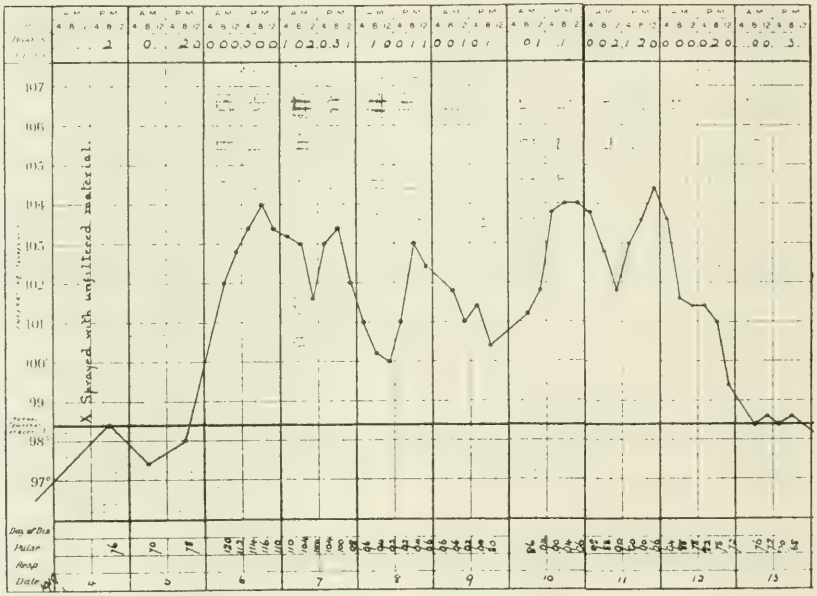
No. 1 and No. 2.—1 c.c. of unfiltered mixture of nasal washings and sputum.

No. 3 and No. 4.—5 c.c. of filtrate.

During the night of March 2, approximately 36 to 40 hours after his instillation of the unfiltered material, No. 1 developed an illness clinically indistinguishable from Influenza. Sudden onset, fever,

severe headache and pains in the back being the prominent features; herpes on the lips and in the anterior nares also appeared on March 4. His temperature chart appears below. He was up for two hours on the sixth day of his illness, and made an uninterrupted recovery.

Chart of No. 1.



None of the other three men were affected by the procedure.

Within an hour of the completion of the instillations in the men two monkeys (*Cercopithecus lalandi*) were similarly treated, as follows:—

Monkey C, with 1 c.c. of the unfiltered material.

Monkey D, with 5 c.c. of the filtrate.

Here, as in the previous instance described, neither monkey was affected.

Chart C (Monkey).

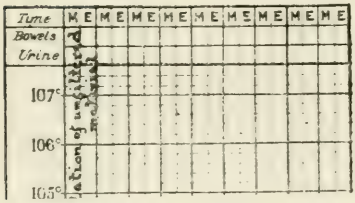
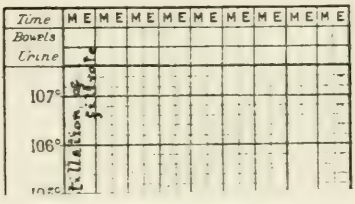
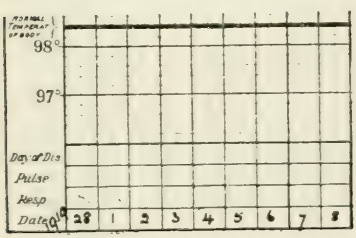


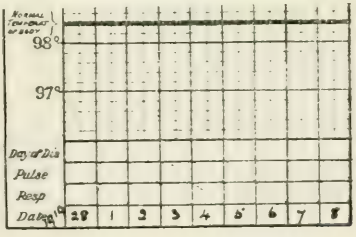
Chart D (Monkey).



ERRATA.

Chart No. 1, on page 406, should be Chart No. 9, on page 409, and *vice versa*.



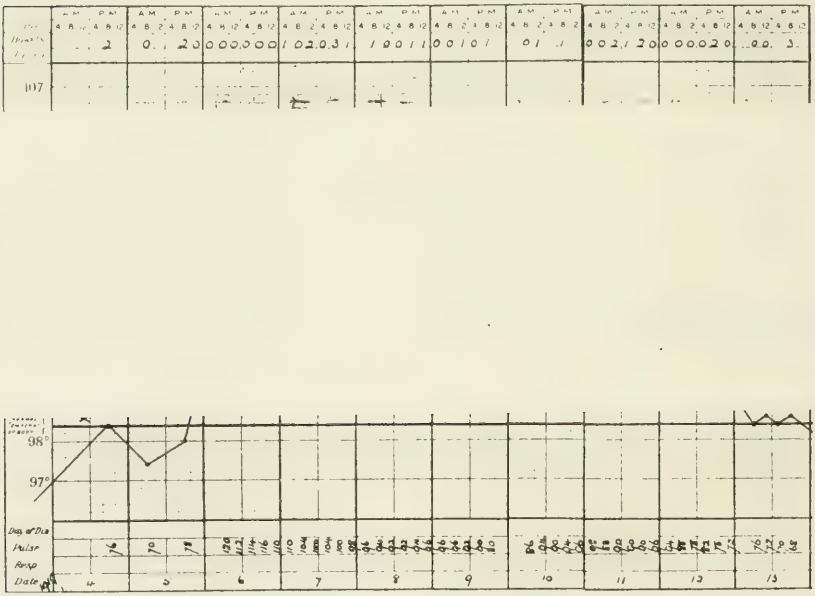


Experiment II.

On March the fourth, a third case of Influenza was made use of, viz, Mr. "Z", age 33 years, a policeman. He attributed his attack to contact with a large number of people in their homes; he was engaged in delivering notices for the payment of rates.

severe headache and pains in the back being the prominent features; herpes on the lips and in the anterior nares also appeared on March 4. His temperature chart appears below. He was up for two hours on the sixth day of his illness, and made an uninterrupted recovery.

Chart of No. 1.



None of the other three men were affected by the procedure.

Within an hour of the completion of the instillations in the men two monkeys (*Cercopithecus lalandi*) were similarly treated, as follows: —

Monkey C, with 1 c.c. of the unfiltered material.

Monkey D, with 5 c.c. of the filtrate.

Here, as in the previous instance described, neither monkey was affected.

Chart C (Monkey).

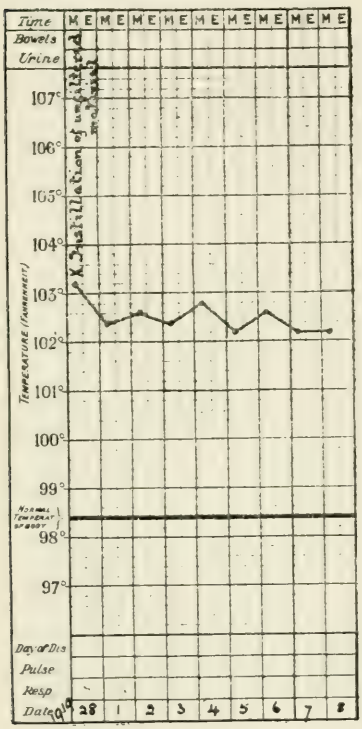
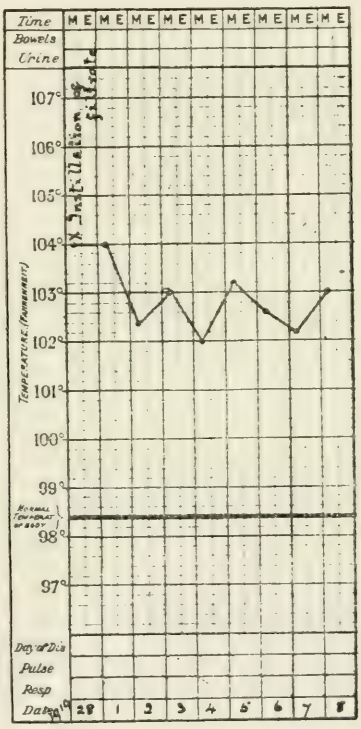


Chart D (Monkey).

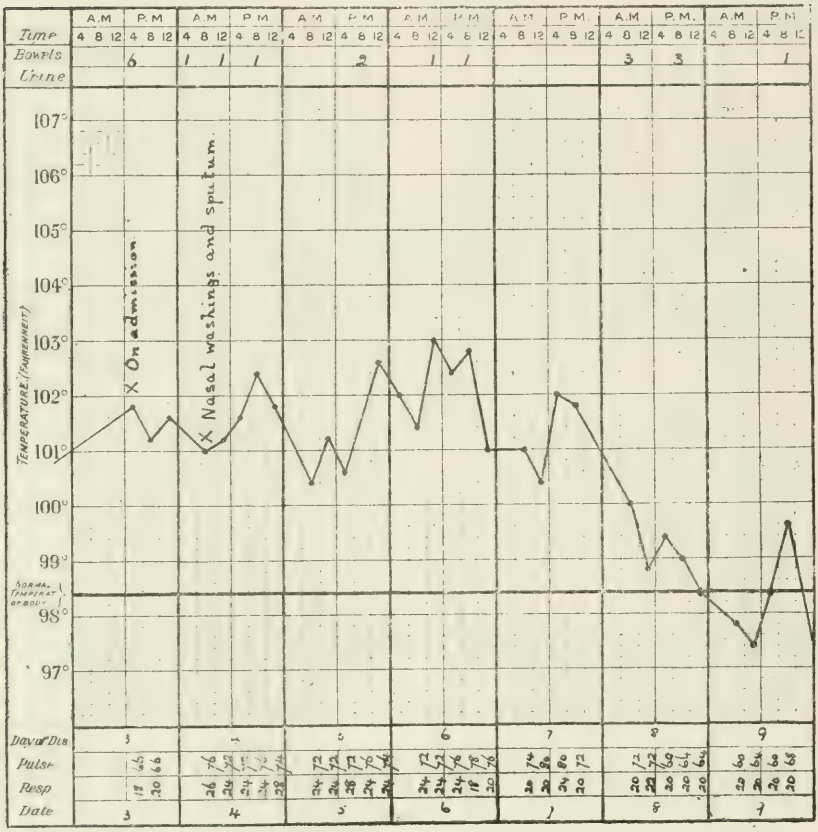


Experiment II.

On March the fourth, a third case of Influenza was made use of, viz, Mr. "Z", age 33 years, a policeman. He attributed his attack to contact with a large number of people in their homes; he was engaged in delivering notices for the payment of rates.

Clinically, his illness appeared to be typically that of Epidemic Influenza. His chart is exhibited below.

Chart of Mr. "Z."



Nasal washings and sputum were collected on *the fourth day* of the lisease in the same manner as described in connection with the two previous cases and were subjected to the same manipulations.

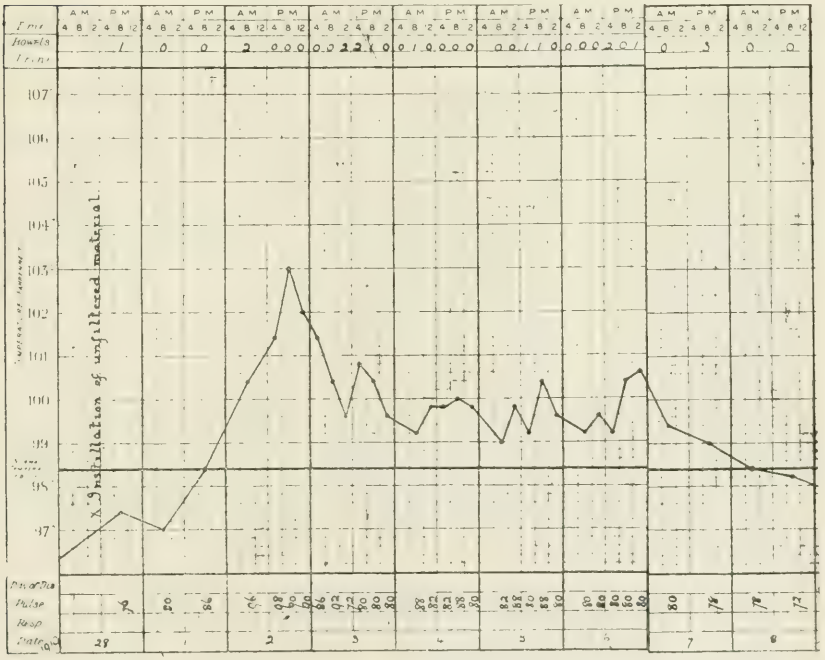
Blood-agar slopes and broth inoculated with the filtrate remained sterile, whilst cultures from the unfiltered material failed to disclose the presence of *Bacillus influenzæ*. Within 1½ hours from the time of the nasal washing the material was transferred to the remaining three untreated volunteers in the following manner:—

No. 9.—1 c.c. of the unfiltered mixture of nasal washings and sputum was sprayed into the anterior nares and mouth by means of an atomizer.

No. 10 and No. 11.—Each of these two men received 5 c.c.'s of the filtrate sprayed into the anterior nares and mouth by means of an atomizer.

During the night of March 6, approximately 36 to 40 hours after his spraying with the unfiltered material, No. 9 developed an illness clinically indistinguishable from Influenza. Sudden onset, fever, headache, backache, perspiration, and pain behind the eyes were the initial features of his attack; pulmonary symptoms developed later, with profuse expectoration of yellowish-green mucopurulent sputum which was at no time even tinged with blood. At this stage he complained of pain in the chest and throat, there was moderate cyanosis, and congestion of the pharynx, but no enlargement of the tonsils. Herpes of the lips appeared on March 14. On March 15 the patient was out of bed for the first time, and thereafter made a good recovery. During convalescence he complained of "noises in his head and ears and a dull heavy sensation in his head." An attempt to cultivate the *Bacillus influenzæ* from the sputum of this case proved a failure. His temperature is shown below.

Chart of No. 9.



Neither of the remaining two men showed any sign of infection. No monkeys were available in connection with this experiment.

Experiments with Cultures.

These experiments comprised attempts to infect the volunteers with cultures of *Bacillus influenzæ*, and also with cultures of two other organisms—one a Gram-negative diplococcus, and the other an extremely small Gram-positive coccus; the colonies of the two latter organisms when grown on blood-agar were very minute. The method used in all these experiments was to remove the growth from the blood-agar slopes by means of a platinum loop, and suspend the organisms in normal saline solution. The number of organisms in these suspensions was not determined, but from their appearance it would not be less than 2,000 millions per c.c. The emulsions were made immediately prior to their being introduced into the noses and mouths of the volunteers by means of an atomizer.

We also sought to infect with *Bacillus influenzæ* the four monkeys which had been previously used in the filtrate experiments.

These procedures will now be described in greater detail.

Experiment III.

For this experiment emulsions of five different organisms were used, and in no case was pooling of organisms resorted to.

The organisms in question comprised three strains of *Bacillus influenzæ* and two unidentified organisms from influenzal cases. Their origin was as follows:—

Three Strains of Bacillus influenzæ.

Strain "P", isolated in the Government Laboratory at Cape Town, October, 1918, from a case of Epidemic Influenza.

Strain "3232," isolated *post mortem*, at the Institute in Johannesburg, October, 1918, from the lungs of a Native who had died of Influenzal Broncho-pneumonia.

Strain "XX," isolated in Johannesburg, October, 1918, from the lungs of a Native who died of Influenzal Pneumonia.

Two unidentified Organisms.

Organism "N."

A Gram-negative diplococcus, morphologically resembling the Meningococcus or *Micrococcus catarrhalis*, but forming extremely minute colonies on blood-agar quite unlike either of the two organisms just referred to. It was recovered from the unfiltered mixture of nasal washings and sputum from Case "Z."

Organism "S."

A very small, Gram-positive coccus, forming on blood-agar minute colonies, but rather larger than those of Organism "N." This organism was obtained at Durban on March 9, 1919, by means of a West swab from the naso-pharynx of Miss D——, on the fourth day of a typical attack of Epidemic Influenza.

The growths from 18-hour-old cultures on blood-agar of these five organisms were separately suspended in normal saline, so that the resulting suspensions were estimated by the unaided eye to contain not less than 2,000 millions of organisms per c.c. Two c.c.s of these five suspensions were immediately sprayed by means of an atomizer into the noses and mouths of nine volunteers, as follows:—

No. 11,	treated with 2 c.c.s of suspension of strain	"3232"
No. 6,	.. ..	"3232"
No. 5,	.. ..	"XX"
No. 7,	,, ,, ,, ,, ,, ,, ,,	"XX"
No. 10,	.. ..	"P"
No. 3,	.. .. organism	"N"
No. 8,	.. ..	"N"
No. 2,	.. ..	"S"
No. 4,	.. ..	"S"

The only individual amongst these nine men who exhibited any definitely recognisable disturbance after these sprayings was No. 6, who, 30 hours later, complained of headache, pains in the shoulders and behind the eyes, and pronounced giddiness and anorexia. He went to bed, where he remained three days, after which he completely recovered. His morning temperature on March 18 rose to 100°F., and his pulse to 106, but these affections of the temperature and pulse were merely transitory, for at 10 p.m. they had fallen to 98°F. and 66 respectively.

Experiment IV.

From the beginning of our investigation we had decided to spray a freshly isolated culture of *Bacillus influenzæ* into the noses and mouths of some of the volunteers and monkeys.

Repeated efforts to obtain such a culture from the Influenza patients at the Beach Hospital and elsewhere were, however, for some time unsuccessful. Various attempts were made with sputum, naso-pharyngeal swabs, nasal washings, a lung puncture (performed on a case five minutes after death from Influenza complicated with Broncho-pneumonia), and the lungs of several fatal cases and the frontal sinus of one such case. Nevertheless we eventually isolated from the lungs of a patient who had died of Influenza with pulmonary complications an organism which, although slightly atypical in the size of its colonies on blood-agar, we felt justified in regarding as *Bacillus influenzæ*.

On human-blood-agar it sometimes produced medium sized (about that of the Pneumococcus) colonies, whilst at other times they were as minute as one is accustomed to see with *Bacillus influenzæ*. These variations could often be seen on the same tube of medium, the upper and drier half of the medium being covered with the minute forms, whilst the lower half exhibited the much larger variety of colony.

Whatever the size of the colonies, when examined microscopically they were found to consist of Gram-negative organisms exhibiting all those variations in size which are commonly characteristic of the *Bacillus influenzæ*.

All cultures planted on one day might consistently show small cocco-bacillary forms; in those planted on another, many long thin bacillary forms would appear, but always together with a number of the smaller variety. A considerable number of the organisms would often show bipolar staining. The organism failed to grow on plain nutrient agar. Although this was the predominating organism found in the lungs of this case, the Pneumococcus was nevertheless present in small numbers.

We used the fourth subculture of this organism in our attempt to infect seven of the volunteers; 1.5 c.c. of a suspension in normal saline was sprayed by means of an atomizer into the noses and mouths of each of the seven volunteers; and the same quantity was instilled by means of a pipette into the noses and mouths of each of the four monkeys.

The suspensions were made immediately before use, and were estimated by the unaided eye to contain not less than 2,000 million organisms per c.c.

No. 5 received 1.5 c.c. of suspension of *Bacillus influenzæ*.
No. 6 ,, ,, ,, ,, ,, ,, ,,
No. 7 ,, ,, ,, ,, ,, ,, ,,
No. 8 ,, ,, ,, ,, ,, ,, ,,
No. 9 ,, ,, ,, ,, ,, ,, ,,
No. 10 ,, ,, ,, ,, ,, ,, ,,
No. 11 ,, ,, ,, ,, ,, ,, ,,

Monkey A received 1.5 c.c. of suspension of *Bacillus influenzæ*.
,, B ,, ,, ,, ,, ,, ,, ,,
,, C ,, ,, ,, ,, ,, ,, ,,
,, D ,, ,, ,, ,, ,, ,, ,,

Neither the men nor the monkeys developed any illness as the result of these sprayings. They were kept under observation for six days. The monkeys' temperatures were recorded for six days, but showed no abnormality. The series of experiments then came to an end.

DISCUSSION.

The period at which these experiments were undertaken was inopportune. The epidemic of Influenza had already passed through South Africa; for this reason all our volunteers, with the exception of Nos. 6 and 9, must have been frequently exposed to infection.

Assuming the correctness of their denial of having been attacked, it seemed probable that they might be definitely resistant to this disease by the natural means of infection. For this reason we felt that only positive results would be of any real value.

It is necessary to record our experience regarding the temperatures of the volunteers; without exception the temperature of these men frequently exhibited variations above 98.4°F. at the recording hour of 6 p.m., the morning readings taken at 6 a.m. being either 98.4°F. or below. Nevertheless the health of the men appeared to be excellent.

We are unable to assign a satisfactory reason for such departure from the normal in respect of temperature. Attention, however, is drawn to the alteration in the environment of the volunteers. The change from six thousand feet above sea level (Johannesburg) to sea level (Durban) was probably not responsible because one of the volunteers whose evening temperature was frequently higher than 98.4°C. was a resident of Durban. The life of the volunteers during their stay on Salisbury Island may be compared to that usually followed at a picnic, viz., absence of work, hearty meals, and a good deal of wandering about, often hatless, under a hot sun. Mosquitos were very numerous, and all the volunteers were frequently bitten. In addition, there may have been some apprehension regarding their risks under the conditions of the experiment.

With regard to the technique used in the filtration experiments—The only departure from common usage was the use of distilled water in the place of normal saline for carrying out the nasal washings; our reason for this preference was that there is some evidence to show that in filtration experiments where Acute Poliomyelitis is concerned distilled water is preferable.

In experiment No. 1, where material from Mr. "X" was employed, the failure to infect any of the four volunteers (two of whom received unfiltered and two filtered material) may indicate that the patient is not always infectious as late as the twelfth day of the disease.

In experiments Nos. I. and II., in connection with the cases of Sister "Y" and Mr. "Z" respectively, one volunteer (No. 1) out of the two treated with unfiltered material from Sister "Y" developed an illness clinically indistinguishable, to us, from Influenza, whilst two others who received the filtrate from this material remained unaffected. Only one volunteer (No. 9) was treated with the unfiltered material from Mr. "Z," and he developed an illness (in approximately the same incubation period of 36 hours, as that of No. 1), which again we could not distinguish from Influenza; whilst two other volunteers who received the filtrate from the same material were not affected.

The material was secured from Sister "Y" and Mr. "Z" on the third and fourth days of the disease respectively.

Little is to be gained by a lengthy discussion of these experiments. Where the experiments were so few in number the impossibility of proving even whether the illnesses of No. 1 and No. 9 were really

Influenza, or whether or not the four volunteers who received the filtrates were naturally resistant, indicates the futility of endeavouring to arrive at any sound conclusion from such meagre premisses.

The filtration experiments on monkeys were entirely negative, and the unfiltered, like the filtered, material gave rise to no illness.

We would emphasise the instability of the temperature of monkeys under even the moderate excitement due to the difficulty sometimes experienced in catching these animals; a rise of two or three degrees could be demonstrated after a few minutes' intentional excitation. The experiments in which cultures of *Bacillus influenzæ* and two unidentified organisms were used proved entirely negative with the exception of the case of No. 6. This man became definitely ill 30 hours after receiving a spray of a suspension of *Bacillus influenzæ*, the particular strain having been isolated four months previously and sub-cultured on human-blood-agar in the interim. This volunteer had not been exposed to infection during the epidemic. He had, however, previously received with impunity the unfiltered material from Mr. "X". We refrain from commenting further on this case, except to state that we are unable to say whether this volunteer's illness was or was not Influenza.

The primary unsuitability of the volunteers, from a scientific standpoint, became more pronounced as each one was submitted to further experiment. In Experiment No. IV., where the remaining seven were sprayed with the recently isolated culture of *Bacillus influenzæ*, six had already been subjected to two previous experiments, and No. 6 and No. 9 had just recovered from the illness already described. This experiment was entirely negative in results. The four monkeys which received the same culture were also unaffected.

SUMMARY.

1. The transference of an unfiltered mixture of nasal washings and sputum from three cases of Epidemic Influenza to the nasal cavities and throats of human volunteers was followed in two instances out of five by illness indistinguishable clinically from Influenza.
2. The incubation period in each case was about 36 hours.
3. The filtrate obtained by passing a mixture of nasal washings and sputum from three cases of Epidemic Influenza through a Berkefeld "N" candle failed to give rise to illness in all of six human volunteers.
4. The monkeys we employed were unaffected by instillations into the nose and mouth of either unfiltered or filtered nasal washings and sputum derived from cases of Epidemic Influenza.
5. The spraying of living cultures of *Bacillus influenzæ* and also two unidentified organisms into the noses and mouths of human volunteers and monkeys was followed by a mild indisposition in one human volunteer only.

It remains to acknowledge our great indebtedness to Dr. H. E. Fernandez, Port Officer of Health, Durban, for many kindnesses and great assistance in connection with the occupation of the Quarantine Hospital by the volunteers. To Dr. P. Murison, Medical Officer of Health, Durban, for allowing us to obtain material from the Beach Hospital. To Lieut.-Colonel Whitehead, A.D.M.S., for the loan of equipment; Captain C. V. Impey for facilities and assistance at the Laboratory, No. 3 General Hospital (Military); to Mr. J. G. Boss, our assistant, and to Mr. H. P. Sheridan, who was in charge of the volunteers throughout.

Finally, both we and the public generally owe a deep debt of gratitude to the individuals who responded to the advertisement for volunteers. The eleven who were selected were prepared to take the risks inseparable from experiments of this nature, and bore themselves with the greatest fortitude and cheerfulness throughout.

April 8, 1919.

TROPICAL ULCER IN NATIVE MINE LABOURERS ON THE WITWATERSRAND.

BY

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AND

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TROPICAL ULCER IN NATIVE MINE LABOURERS ON THE WITWATERSRAND.

By F. S. Lister and H. Q. F. Thompson.

Through the work of Vincent,[1] Prowazek,[2] and others it is now generally admitted that Tropical Ulcer is caused by the *Bacillus fusiformis*, and a spirillum or spirochaete. The association of these two organisms is either an example of symbiosis, or else the spirochaetes are derived from the *B. fusiformis*; the work of Tunnicliff[3] and others points to the latter view as the more probable.

One of us (F.S.L.)[4] drew attention to the occurrence of these ulcers in mine Native labourers in 1911, and described their bacteriology and a method of treatment. From time to time extensive epidemics of Tropical Ulcer arise amongst the Natives on the gold-mines of the Witwatersrand, and on the coal- and gold-mines of Southern Rhodesia; in 1910-11 a formidable outbreak occurred on the Premier Diamond Mine. Although permanent disablement is a rare event in this disease, the economic wastage due to the loss of working time is a very serious matter.

Early in the present year, *i.e.*, in the height of summer, it was brought to the notice of the Institute that on certain of the local gold-mines there were very many Natives suffering from grievous sores, mainly on the lower extremities, and which showed little tendency to heal. These sores, in a large percentage of the cases, extended by a process of phagedaenic ulceration and led to a lengthy period of disablement. We have investigated a number of these cases, and the results of our work are reported in this paper.

The investigation comprised:—

(1) Clinical Examination.
(2) Bacteriological Examination.
(3) Histological Examination.
(4) Treatment.

1. Clinical Examination.

At a preliminary examination of a collection of cases in one of the mine hospitals, it became at once apparent that the lesions had not usually originated in the manner described in certain text-books. For instance, in Castellani and Chalmers' "Manual of Tropical Medicine," p. 1561, *Ulcus tropicum* is said to begin "with the appearance of a small, painful, occasionally pruriginous papule, or papulo-pustule, surrounded by a deeply infiltrated, dusky, red areola." The lesions which formed the subject of our preliminary examination

were, with but one exception, the outcome of traumatic breaches of the skin, and in nearly every instance the original wounds had been caused by pieces of rock falling on to or against the lower limbs.

The ulcers were, as a rule, solitary; occasionally they were multiple, varying from two to five in number and being situated either on the same or on different limbs. The majority were circular or oval in outline (with a diameter of from ·5 to 7·5 cms.), though irregular shapes were to be met with. In some instances the ulcers were deep and crateriform, in others shallow; the edges were sometimes thickened and often undermined; the base was usually covered with greeny-yellow pus, frequently possessing an acrid and unpleasant odour; after removal of the pus the floor of the ulcer was seen to be covered with granulation tissue which was at times exuberant, but often poorly developed; haemorrhage occurred very readily on probing the undermined edge or the soft base of the ulcer. In some cases, tendons had been exposed, and the destruction of the tissue had occasionally extended to the underlying bone resulting in a localized caries or in the formation of a sequestrum. The ulcers were practically always situated on the lower limbs, and below the knee in almost every case; the most common sites were the anterior surface of the leg, the dorsum of the foot, over the malleoli, and in the area overlying the *tendo Achillis*.

The first question to be determined was whether the lesions were merely wounds infected with the ordinary pyogenic micro-organisms, or whether they were—as we suspected—examples of infection with the *B. fusiformis* and its associated spirillum. In all cases the original wound had undergone enlargement and had assumed all the characters of a lesion which might be legitimately described as an ulcer.

2. Bacteriological Examination.

Seventy-three cases were bacteriologically examined. Of these, four were in Native employees of the Southern Rhodesian Railways and the remaining sixty-nine were in Native labourers on the gold-mines of the Witwatersrand. Forty cases were culturally examined, thirty-one with a view to isolating the various organisms present, and nine in an attempt to grow the *B. fusiformis*. Attempts to infect rabbits, guinea-pigs, and mice by scarification and the application of material from the ulcers proved ineffective, no lesion at all being produced.

Material from the floor and the undermined edges of the ulcers was examined bacterioscopically in each of the seventy-three cases. The routine method of staining was both by dilute carbol-fuchsin and by Gram's method. In addition, a number of slides were stained both by Leishman's method and by silver impregnation, whilst others were examined directly by dark-ground illumination. Among these seventy-three cases the *B. fusiformis* was present in thirty-eight, but the spirillum (always in association with the *B. fusiformis*) was observed in nine only, which number included the four Rhodesian cases.

A certain number of the ulcers in the above series were investigated at a time when the healing process was well established, and it is therefore probable that the *B. fusiformis* would have been found in a greater proportion had these been examined at an earlier stage.

All attempts to grow the *B. fusiformis* ended in failure. The media used were human-blood-agar, horse-serum broth, and trypsinated broth. The culture tubes were incubated anaërobically, the air having been removed by means of an air-pump and subsequent washing with hydrogen. The nine cases in connection with which these attempts were made were selected on account of the purity and abundance of the infection with the *B. fusiformis*, as disclosed by bacterioscopic examination.

The results of the general cultural examination of the thirty-one cases referred to above are recorded in Table I.; the culture medium used was human-blood-agar, and aërobic methods alone were employed.

TABLE I.

Micro-organisms isolated by aërobic Cultivation.

No. of Cases examined.	Haemolytic Streptococci (in pure culture).	Haemolytic Streptococci with *B. proteus*.	*B. proteus* (in pure culture).	*Staphylococcus albus* (in pure culture).	Various unidentified micro-organisms.	No growth in tubes.
31	13	3	3	4	6	2

In this series of thirty-one cases, the *B. fusiformis* had been found, bacterioscopically, to be present in eleven, and in four of these, but in no others, the spirillum was also present. It will be noticed that in the remaining forty-two cases the *B. fusiformis* had been detected in twenty-seven instances. The explanation of the relative infrequency of its occurrence in the series recorded in Table I. probably lies in the lack of selection in the cases, which included those that had been subjected to much treatment, and, moreover, a number in which the ulcers were already in the healing stage. The series comprised, in fact, the first thirty-one patients who came under observation, whereas the remaining cases were more carefully selected in order to obtain satisfactory examples for testing the various forms of treatment that we had in view.

In the eleven infections with *B. fusiformis* the *Streptococcus haemolyticus* was also present in nine, and the *B. proteus* in two instances.

Fourteen of the sixteen strains of the *Streptococcus haemolyticus* produced acid in both lactose and salicin, but failed to ferment mannite; of the remaining two strains, one failed to ferment lactose

and mannite but produced acid in salicin, whilst the other did not ferment any of the three mentioned substances.

The six strains of *B. proteus* conformed to the type of *Proteus vulgaris*. A small quantity of culture planted at the bottom of a tube of nutrient agar spread upwards and reached the top in twenty-four hours. All the strains were actively motile, and showed the presence of peritrichic flagella, some bacilli possessing from 20 to 25.

It was thought worth while to carry out the Weil-Felix reaction with the sera from the cases in which *B. proteus* was present. Six cases of ulcer showing a local infection with *B. proteus*, and six others in which this micro-organism was absent, were selected for this purpose. Two strains of *Proteus X*—both obtained from the Lister Institute—and the six locally acquired strains of *B. proteus* already referred to, were separately tested. The dilutions of serum used were 1/20, 1/40, 1/80, and 1/160. The agglutination mixtures were incubated at temperatures of 37°C. and 55°C. for 2- and 24-hour periods. There was not the slightest trace of agglutination in any of the preparations.

The Wasserman test was performed in twelve typical cases, with a negative result in all except one; in this instance a weakly positive reaction was obtained. These results appear definitely to exclude tertiary Syphilis from any share in the production of these lesions.

Leaving out of account the four Rhodesian cases, in which the *B. fusiformis* was constantly associated with a spirillum, it is to be noted that there were thirty-four cases from the Rand which showed the presence of *B. fusiformis*, but that in only five of them was the spirillum also observed. This was an unexpected finding, and one which was not at all in conformity with the previous experience of one of us[4] during the very severe outbreak on the Premier Diamond Mine already referred to; on that occasion the spirillum was found to be consistently associated with the *B. fusiformis*. We have no explanation to offer for this divergence on these two occasions. The ulcers which occurred on the Premier Diamond Mine were far more intractable, and caused greater and more rapid destruction of tissues, than those which we have recently studied on the Witwatersrand, and it may be surmised that the presence of the spirillum determines a more serious form of lesion.

3. Histological Examination.

Material sent to us from five cases in the City Deep Mine Hospital was examined by Dr. J. H. Harvey Pirie, of the Routine Division of the Institute, and the following observations were made:—

"In four instances the material consisted of scrapings from ulcers, in the fifth case the whole ulcer had been excised and submitted for examination.

TABLES II

Showing Treatment and Clinical Cures classified to one of three different

Methods of Treatment

(Where the bacteriological diagnosis was not made at the time the *B. tuberculosis* had been found bacteriscopically, the specimen was examined in Case 2,262-end.)

Case No.	Age	Situation of Disease	Approximate Amount of Disease in inches	Nature of Initial Injury	Course of Disease	Number of days from beginning of treatment to complete healing of Ulcers
				Group I.—Treated by Excision		
1,834	Female, Middle-aged	Over the middle third of anterior aspect of tibia	4·0	Struck by a pointed rock	None	64
1,960	Female, Elderly	Over the lower 1/3 of the right tibia and internal malleolus	2·0	Struck by a pointed stone	None	54
2,196	Female, Middle-aged	Over the middle third of left tibia	4·0	Struck by a pointed rock	None	121
2,211	Female, Middle-aged	Over the middle third of anterior surface of right leg	4·0 × 3·0	Struck by a pointed stone	None	103
2,344	Female, Elderly	Covering all the front of anterior surface of both legs	20·0 × 2·0	Struck by a pointed rock	Sore remained open for Twelve Months	Still unhealed after 365 days
2,9[illegible]	[illegible]	Over the front of the lower third of the right leg	3·0 × 2·0	Struck by a pointed rock	Leg broke out in boils with discharge of pus from sore	Still unhealed after 164 days
				Group II.—Treated by Curetting		
1,[illegible]	Male	[illegible]	[illegible]	Struck by a pointed rock	None	40
1,[illegible]	[illegible]	[illegible]	4·0	Struck by a pointed stone	None	68
1,[illegible]	[illegible]	[illegible]	2·0	Struck by a pointed rock	None	46
1,[illegible]	Female	[illegible]	3·0 and 1·5	Struck by a pointed stone	One other sore which had existed for 14 years was not healed after	74
1,[illegible]	[illegible]	[illegible]	2·0 × 1·5	Struck by a pointed rock	None	70
1,[illegible]	Male	[illegible]	[illegible]	[illegible]	Ulcer broke out again after healing	Still unhealed after 274 days
				Group III.—Treated by Local Applications Only		
1,[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	None	39
1,[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	None	57
1,[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	None	45
1,[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	None	43
1,[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	None	59
1,929	[illegible]	[illegible]	[illegible]	[illegible]	None	51

"The material comprising the scrapings proved, in two instances, to be very necrotic and haemorrhagic, and little could be made out histologically. The other two showed granulation tissue similar in type to that found in the fifth case, but as the positional relationship to the ulcer as a whole was indeterminable no further description need be given.

"In the fifth case it was possible to cut sections which were definitely orientated as regards the edge and floor of the ulcer. The ulcer, to the naked eye, appeared to be one which was healing rather than extending; the periphery was thickened and a fresh growth of young epithelium was extending inwards towards the centre. The floor in the central part showed reddish granulations with only a comparatively small amount of patchy fibrinous membrane.

"Microscopically, the floor of the ulcer showed granulation tissue of the ordinary type, with moderately abundant polymorphonuclear leucocytes in the surface layer; in the deeper parts, lymphoid and plasma cells dominated the picture. This gradually merged into the noninfiltrated fibrous tissue of the subcutaneous layers, but, both below the floor and laterally for a considerable distance beyond the edge of the ulcer, there were to be seen amongst otherwise healthy tissue streaks and focal collections of mononucleated cells, suggesting that the inflammatory process had extended along lymph channels or in tissue spaces for some distance away from the actually ulcerating area. Eosinophilous cells were not uncommon among the round cells, but they did not appear to be specially congregated around vessels, as has been described by some writers.

"The general picture was sufficiently in accordance with the text-book descriptions of Tropical Ulcer. Sections were not stained with special reference to determining the distribution of the spirochaetes and fusiform bacilli respectively."

4. Treatment.

It was our intention to investigate a number of different methods of treatment for this disease; before the work could be completed, however, the outbreak ceased. This occurred when only three procedures had been applied to three different groups of six patients each. The failure in the supply of fresh cases was particularly disappointing, as it prevented us from trying the effects of ionization, trypan blue, etc.

The three forms of treatment investigated were:—

Group (1)—Excision of the ulcers.
Group (2)—Curetting of the ulcers.
Group (3)—Local application of novarsenobillon.

The six cases in each group were not entirely comparable with one another, nor with those in the other groups. In two cases in group 1 the ulcers involved the underlying bone, with the formation of a sequestrum in each case. In two cases in group 2, also, the ulcers

involved the bone, and in one of these the medullary cavity had become infected and a sequestrum had formed. In group 3 none of the ulcers extended to the bone, although five of them were 2·5 cms. or more in diameter. In each group there was one individual who was affected with more than one ulcer.

A variety of applications was used in the subsequent treatment of those ulcers which were submitted to excision and curetting, including saline, tincture of iodine, acro-flavine, aluminium acetate, and "red lotion" (2 grs. of sulphate of zinc, 12 minims of compound tincture of lavender, with water to one ounce). In the group treated by novarsenobillon the only subsequent application was hydrogen peroxide. Table II. records the chief points of interest in the individual cases of each group.

The results noted in Table II. indicate that the treatment of these ulcers by the local application of novarsenobillon was at least as efficient as the more severe procedures of excision or curetting. Equally good results were obtained by applying the drug either in the form of a powder or in solution. In cases in which the lesion involved the bone surgical measures were necessary in all instances.

The number of remedies which have been advocated for Tropical Ulcer is very large, and this fact alone is evidence of the unsatisfactory position as regards the treatment of this lesion to-day. Series of cases have been reported[6] in which a cure was effected in fifteen days, even when the loss of tissue had been considerable. Such successes are outside our experience and lead us to speculate concerning the correctness of the conception of Tropical Ulcer as a clinical entity.

Discussion.

It will be seen from Table II. that this disease affected Native labourers on the Rand who had been recruited from tribes located so far apart as the Cape and Rhodesia; it may therefore be concluded that there is no tribal immunity from it. As to the possible existence of any racial immunity, we have no direct evidence. It may, however, be recorded that we have not seen or heard of a case of the disease in a European, either at the Premier Mine, or on the Rand. This apparent freedom may possibly be associated with the protection to the lower limbs which the wearing of European clothing secures. The history of an injury, antecedent to the appearance of the ulceration and usually caused by a fall of rock, was almost constant; only one exception appears amongst the eighteen cases in Table II. Another noteworthy fact is the special incidence of the disease upon Natives working underground, the disease, indeed, appears to be confined almost entirely to this class of mine labourer. At first sight, this discrimination might be thought to point to the underground workings themselves as being the essential source of the infection. It is probable, however, that this is not the case, and that employment underground is merely a predisposing cause. It has long been recognized that Lobar Pneumonia, as it occurs on the Rand, is

relatively far more common amongst the underground than the surface employees, and one attributes this peculiarity, not to any saprophytic prevalence of the Pneumococcus in the workings, but to the predisposing effects of exhausting work, hot, humid atmospheres, and somewhat sudden changes of temperature. If these things are true with respect to pneumococcal infections they are probably also true with respect to Tropical Ulcer. Castellani[5] states that "there are localities, however, such as the hinterland of Aden, the low marshy plains of Cochin-China and Tonkin, and some islands of the Red Sea where the affection is particularly common." This points to hot, damp atmospheres being closely associated with the infection, and the underground workings of the gold-mines reproduce these conditions far more closely than do the surface workings. The number of injuries to the lower limbs amongst underground labourers is, moreover, out of all proportion to the number of such injuries amongst workers on the surface. This and the other considerations mentioned suggest that the infection is not necessarily derived directly from the underground workings.

In 1911 one of us[4] suggested that the patient might owe the specific infection of his wound to his sputum or nasal discharge, and some confirmation of this possibility is forthcoming in the recent work of Reckford and Baker[7], in connection with Vincent's Angina infection; these workers occasionally found Vincent's micro-organism in normal mouths, and in 90 per cent. of diseased teeth.

It has been suggested by some that these ulcers, as seen on the Rand, originate in the hospitals from a transference of the infection from established cases of the disease to previously non-infected wounds. That this has happened in some instances is highly probable, but there is evidence to show that it is not always, or even usually, the explanation. Practically all the cases comprising the outbreak on the Premier Diamond Mine in 1910-11[4], and all those in the recent epidemic which were dealt with by the medical administration of the City Deep Mine, were first admitted to hospital with the ulceration already established.

When these ulcers become prevalent the economic loss entailed is very great; the number of working days wasted is sufficiently evidenced by the record of the eighteen cases in Table II. A loss of as many as 4,000 days with respect to twenty-two cases is within our experience.

The most satisfactory way of dealing with this disease on the mines is to prevent, as far as possible, its occurrence. This would be secured by antisepticizing all wounds, no matter how trivial, at the earliest possible opportunity, and submitting all such cases to daily inspection until the wound is healed. The earliest indication of the onset of phagedaenic ulceration should be met by the local application of novarsenobillon. When once the lesion has become well established it is obvious that, even if the infection could be promptly eradicated from it, many days or possibly weeks must elapse before its healing can be completed.

Conclusions.

(1) That in a recent epidemic of ulcers, occurring almost solely in Native mine labourers working underground, Vincent's fusiform bacillus was frequently present.

(2) Vincent's spirillum was usually absent.

(3) Haemolytic streptococcus and *B. proteus* were isolated in a number of instances.

(4) Treatment by local application of novarsenobillon was found to be as effective as excision or curetting of the ulcers.

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Tropical Ulcers in a Native Mine Labourer.

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